

**Garlic Mustard (*Alliaria petiolata*) Invasion & Impacts:
implications for management and restoration of woodland herbs**

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Laura Phillips-Mao

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Diane L. Larson & Nicholas R. Jordan

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Abstract

The invasion of the biennial herb garlic mustard (*Alliaria petiolata*) in North American woodlands has coincided with declines in native plant communities, motivating the question: is garlic mustard driving or responding to ecosystem change? Garlic mustard can affect soil chemistry and arbuscular mycorrhizal fungi (AMF) communities; the extent to which such impacts contribute to woodland degradation will affect the outcomes of invasion control efforts, including biocontrol. Post-invasion plant community recovery is unlikely if garlic mustard is not a primary driver of native plant decline, or if soil legacy effects persist following garlic mustard's removal. In this study, I investigated the impacts and legacies of garlic mustard and the implications for restoration of woodland herbaceous communities in Minnesota. Specifically, in a combination of field and greenhouse studies, I tested the hypotheses that native herbs would have lower germination, establishment, biomass, and mycorrhizal colonization when planted into invaded soils compared to non-invaded soils, and that such impacts would persist after multiple years of complete or partial removal of garlic mustard, indicating a soil legacy effect. Further, I tested the hypothesis that garlic mustard invasion is responding to native herb decline by comparing the performance of garlic mustard plants seeded into field plots of varying species richness and native cover.

In two oak woodland study sites, I planted 12 species of native woodland herbs (plug or bare rootstock) into invaded and non-invaded plots subjected to vegetation removal treatments that varied in their degree (full, partial and no removal) and duration prior to planting (two, one or no years of removal). I measured plant biomass after two or three years to test the impacts of garlic mustard presence and history relative to that of non-invaded native vegetation, as well as the persistence of garlic mustard's impacts at low densities or following sustained removal. I tested mechanisms of impact by comparing AMF colonization, and light and nutrient availability in invaded and non-invaded areas. Garlic mustard presence and history did not negatively impact herb biomass, but instead had a facilitative effect resulting in higher biomass in invaded plots. Light availability

and AMF colonization were not affected by invasion relative to native vegetation, but soil resource availability was higher in invaded plots. Herb biomass and nitrate availability remained elevated following multiple years of removal, indicating a “positive legacy” due to nutrient enrichment.

In the greenhouse studies, I tested garlic mustard’s impacts on germination, growth and AMF colonization of 13 native herbs planted by seed into field soils collected from invaded and non-invaded areas. Additionally, I included a fungicide treatment to more explicitly test the AMF mechanism of impact. I found that while garlic mustard did reduce seed germination and mycorrhizal colonization of native herbs, the effects on seedling establishment (herb biomass) varied. Herb biomass was lower in invaded soils in the first experimental replication, when plants were inadvertently subjected to climate stress, and higher in invaded soils in the second replication, under controlled climate conditions; the inconsistent response suggests that garlic mustard’s negative impacts on herbs only manifest when combined with additional stressors.

To investigate whether garlic mustard invasion responds to native herb decline, I planted garlic mustard seeds into field plots that ranged in species richness and cover and measured the direct and indirect effects of native plants and light availability on sequential life stages of garlic mustard. Light levels had varying impacts on different garlic mustard life stages, resulting in net negative effects on garlic mustard numbers but positive effects on reproductive output per plot. Native plant cover had a significant direct negative effect on all garlic mustard life stages, and native species richness had indirect negative effects mediated through higher plant cover in species-rich plots.

Together, the results of these studies support the characterization of garlic mustard as a “back-seat driver” of change in woodland systems; initial invasion appears to respond to declines in native herbs, and once established, garlic mustard’s impacts on native herbs are generally positive—apparently driven by nutrient enrichment—except perhaps under conditions of stress. Long-term population dynamics of herbs in invaded woodlands may be impacted by reduced germination and interactions with multiple stressors. However,

the absence of a negative immediate or legacy effect on herb biomass suggests that native herb restoration will not be inhibited by garlic mustard, although mitigation of additional stressors and primary drivers of change will likely be required for successful restoration outcomes.

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Chapter 1

Beyond Invasion: implications of impacts and legacies for post-invasion restoration

Drivers or Passengers? Implications for Restoration

Managing invasive plant species is one of the most significant and costly challenges of native plant community restoration. Substantial investments of time, labor, and financial resources are required to prevent invasions, contain their spread, and reduce their densities once established (Pimentel et al. 2005). The outcomes of invasion management are not certain, however, and do not always lead to recovery of the invaded system (Reid et al. 2009, Suding 2011). Moreover, such investments may not be sustainable (Larson et al. 2011), particularly given the numerous other threats to plant communities and the expected increase in invasions due to globalization and climate change (Meyerson and Mooney 2007, Hellmann et al. 2008, Dukes et al. 2009). Strategic allocation of management resources, informed by better understanding of invasion impacts and interactions, is necessary to keep pace with growing threats to native plant communities, and to develop comprehensive and effective restoration plans. A central question that must be grappled with is: do the threats of invasion and outcome of invasion control warrant the investment? If the answer to either part of this question is “no”, we may be throwing away resources that could otherwise be directed toward more effective restoration activities. Improving post-invasion restoration outcomes requires a more nuanced understanding of both the various pathways by which an invader alters the system, and the state of the system following invasion control.

The impacts of invasive species on native plant communities—and the outcomes of their control—are largely driven by the mechanisms of invasion (Levine et al. 2003) and the nature of the interactions between the invader, the biotic and abiotic components of the ecosystem, as well as interactions with other environmental stressors and agents of

change. In other words, management outcomes may differ depending on whether a given invasive species is driving or responding to environmental change (MacDougall and Turkington 2005, Bauer 2012). Three models of invasion have been proposed to address this question and explore the implications for management and restoration. MacDougall and Turkington (2005) first characterized the “driver” and “passenger” models of invasion in a study that investigated whether exotic grasses achieved dominance in oak savannas of British Columbia, Canada through competitive mechanisms, thus directly driving declines in native species, or as a result of non-interactive processes that facilitated invasion at the expense of native species, specifically fire suppression. These models, and their implications for management, were further developed by Bauer (2012), who also proposed a third model, the “back-seat driver”, for species that did not fall at either extreme of the driver – passenger continuum.

Drivers are invasive species that introduce a new trait or process to an ecosystem, thereby fundamentally altering ecosystem attributes in a manner that inhibits native species and often facilitates continued reinvasion through positive feedbacks (MacDougall and Turkington 2005). Declines in native species can be directly attributed to the effects of invasive drivers, and therefore effective invasion control is necessary, and potentially sufficient, for ecosystem restoration, provided invasion legacies do not persist (Bauer 2012, Corbin and D'Antonio 2012). It has been proposed that drivers, a category that includes “ecosystem transformers”(Richardson et al. 2000) and “invasive ecosystem engineers” (Jones 1997, Cuddington and Hastings 2004), should be prioritized for prevention and management, given their strong and potentially irreversible ecosystem impacts (Richardson et al. 2000, Richardson et al. 2007, Bauer 2012).

Invasive passengers, on the other hand, achieve dominance by taking advantage of disturbances that decrease the abundance or diversity of native species (MacDougall and Turkington 2005). They may be responding either directly to declines in native species, benefiting from the unutilized resources or vacated niche (Davis et al. 2000, Shea and Chesson 2002), or they may be facilitated by the same processes or disturbances that suppress native species (MacDougall and Turkington 2005). In either case, removal of

the invader is not expected to lead to community recovery, but may instead further contribute to ecosystem disturbance (Bauer 2012). Ecosystem restoration will require identification and mitigation of the disturbances causing the apparent displacement of natives by exotics. Without such mitigation, invasion management may be unwarranted, outcomes of post-invasion restoration will be unsatisfactory—and considerable resources may be wasted in the effort.

The “backseat driver” model was proposed to describe invasive species that express both driver and passenger characteristics (Bauer 2012). Like passengers, these invaders are facilitated by environmental stressors that inhibit native species, but upon invading, they continue to modify ecosystem traits and further contribute to native species decline. In this sense, they are both driving and responding to environmental change, and therefore ecosystem restoration must be comprehensive, including both invasion control and mitigation of the underlying causes of invasion and degradation of the native plant community (Bauer 2012).

Management of invasive species that either cause or contribute to native species decline will likely continue to be a substantial aspect of restoring ecosystems and conserving biodiversity. Distinguishing drivers (backseat or otherwise) from passengers will allow for more efficient and targeted allocation of the very limited resources available for management and restoration. But effective post-invasion restoration also requires an understanding of the mechanisms of impact (Levine et al. 2003), and the potential legacies of invasion that may continue to inhibit recovery and restoration following invasion control (Corbin and D'Antonio 2012). Biotic legacies such as dispersal and recruitment limitation often result when native species populations have been depressed for extended periods of time (Seabloom et al. 2003, Corbin and D'Antonio 2004, Standish et al. 2007, Brudvig et al. 2011), regardless of the mechanism of impact. Invasive seed banks and propagule pressure may exacerbate this effect (Reinhardt Adams and Galatowitsch 2008), but overcoming such biotic legacies with invasion management and species additions is common practice in ecological restoration. Soil legacies, on the other hand, present a broader array of relatively untested challenges

for restoration. Invasive species that alter the chemical, physical and biotic attributes of soil systems may have long-lasting impacts that persist beyond invasion control (Corbin and D'Antonio 2012), continuing to hinder both natural recovery and active restoration. The nature of such legacies, how long they persist, and whether they can be actively reversed all have important implications for restoration strategies and outcomes.

Identifying the degree to which an invasive species drives or responds to environmental change is critical for developing comprehensive, cost-effective and sustainable restoration plans. Doing so, however, requires careful experimentation; because the outcome of all three models is the same—natives decline, invasion expands—we cannot discern the process behind the pattern without explicitly manipulating and testing the interactions of native and invasive species and other potential agents of change (Bauer 2012). Furthermore, the position of a given invasive species on the driver – passenger continuum may vary regionally or depend on site- or community-specific conditions. Thus, as always in science, repeated testing of these models for a given invasive species across a range of plant communities, geographic locations and edaphic and climatic conditions will yield a more robust understanding of the inherent nature of the invader, while also informing more nuanced site-specific approaches to invasion management and restoration. This nuanced approach may be particularly important for developing proactive restoration approaches and long-term adaptive management plans that remain relevant in a changing climate (Hobbs and Cramer 2008).

Research Context and Objectives

As the focus of my PhD research, I explored the impacts and legacies of the invasive herb garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] and the implications for restoration of woodland herbaceous communities in Minnesota. Garlic mustard is often characterized as a driver of change in woodland understories (Nuzzo 1999, Scott 2000, Meekins and McCarthy 2002, Evans and Landis 2007, Rodgers et al. 2008a), and

its soil-mediated mechanisms of impact raise concerns about legacy effects (Stinson et al. 2006, Callaway et al. 2008, Rodgers et al. 2008b). Listed as a prohibited noxious weed in Minnesota in 2003, garlic mustard has achieved a relatively high profile in the past decade, as land managers and property owners struggle to keep pace with its rapid spread, and ecologists investigate its “novel weapons” (Callaway et al. 2008). Meanwhile, native woodland herbs appear to be declining (Brewer 1980, Robinson et al. 1994, Rooney et al. 2004, Rooney and Rogers 2011), and a suite of widespread and potentially synergistic environmental stressors may be responsible (Meier et al. 1995, Drayton and Primack 1996, Jolls 2003, Bohlen et al. 2004, Côté et al. 2004, Flinn and Vellend 2005, Wiegmann and Waller 2006, Nuzzo et al. 2009, Greene and Blossey 2011, Hahn and Dornbush 2012). Placing the relative impacts of garlic mustard into the broad context of woodland decline is necessary to develop a sound strategy for woodland restoration. Bauer (2012) characterized garlic mustard as a “back-seat driver” of change in North American woodlands, which suggests that controlling garlic mustard will not be sufficient for restoration of native plants.

Currently research is underway to develop a biocontrol program for garlic mustard (Gerber et al. 2009), and—pending approval by the USDA/APHIS Technical Advisory Group—a root-crown mining weevil, *Ceutorhynchus scrobicollis*, will be test-released in six Minnesota woodlands (Van Riper et al. 2010). This further highlights the need to understand the magnitude and duration of garlic mustard’s impacts. Not only is it necessary to document impacts to justify the risks and research investment associated with biocontrol (Blossey et al. 2001b), but it is important to anticipate the likely community response to biocontrol and develop proactive restoration plans. If garlic mustard is indeed a “backseat driver” of change, potential legacy effects and primary causes of woodland degradation may need to be factored into woodland restoration.

Against this backdrop of growing concern about garlic mustard and upcoming biocontrol trials in Minnesota, I developed a research program to explore the following questions: Is garlic mustard driving or responding to declines in Midwestern woodland herbaceous communities? Do garlic mustard’s impacts persist at low densities, such that

communities might remain affected following biocontrol? More specifically, does garlic mustard's disruption of arbuscular mycorrhizal fungi (AMF) leave a soil legacy effect that will inhibit recovery and restoration? And what are the implications of such legacy effects for timing and methods (e.g. seeds vs. plants) of woodland restoration?

The study sites for this research are both potential test-release sites for forthcoming biocontrol (Van Riper et al. 2010), and as such, this research is designed to complement monitoring of invaded plant communities before and after biocontrol is initiated. In Chapter 2, I present a field study in which native woodland herbs were planted into invaded and non-invaded plots subjected to vegetation removal treatments to test the impacts on herb growth and mycorrhizal colonization, including potential legacy effects and persistence at low densities. Although the AMF-mediated pathway of impact was the primary focus of this research, I also explored potential impacts via altered resource availability. Chapter 3 describes a greenhouse study in which native herb seeds were planted into field soils collected from invaded and non-invaded areas to test the soil-mediated impacts on germination, establishment and mycorrhizal colonization of seedlings. The greenhouse experiments build on the field study in chapter 2 by including a fungicide treatment to more explicitly test the AMF mechanism of impact, and by investigating garlic mustard's impacts on seed and seedling life stages of woodland herbs. Understanding the impacts of garlic mustard on different life stages can inform both restoration strategies and predictions of recovery and long-term population trends in invaded systems. While the studies described in chapters 2 and 3 test the hypothesis that garlic mustard is driving declines in native herbs, in Chapter 4, I investigate whether garlic mustard invasion in fact responds to such declines. I present a field study in which I planted garlic mustard seeds into plots that varied in species richness and cover and measured the direct and indirect effects of native plants and light availability on sequential life stages of garlic mustard. Together, the goal of these studies was to anticipate likely outcomes of garlic mustard biocontrol and post-invasion restoration of woodland understories.

Chapter 2

The Legacy of Invasion: effects of garlic mustard (*Alliaria petiolata*) and its removal on native herb restoration

The nature and mechanisms of plant invasion impacts have important implications for management outcomes and post-invasion restoration of plant communities. Failure of native communities to recover following invasion control may be due to insufficient control levels, invasion legacy effects, dispersal limitation, or because other environmental stressors are driving declines in native plants and preventing recovery. Garlic mustard is an invasive biennial herb that appears to impact woodland communities via multiple mechanisms; removal of garlic mustard often does not lead to native plant recovery. In this study, I investigated the impact of garlic mustard on native herbs, the potential mechanisms of impact, and whether the impacts depended on the presence of living garlic mustard or persisted as a soil legacy effect. I further tested whether the impacts persist at low garlic mustard densities (i.e. incomplete removal), and whether they persisted following multiple years of garlic mustard control. Twelve species of native woodland herbs were planted into invaded and non-invaded field plots to which various garlic mustard removal treatments were applied. Impacts were assessed by measuring above-ground biomass two and three years following planting. Mechanisms examined included root colonization by AMF and availability of light and soil resources. Garlic mustard presence did not negatively impact herb biomass as hypothesized; in many cases herbs had higher biomass in invaded plots than in non-invaded plots. Light availability and root colonization by AMF were not affected by garlic mustard relative to native cover, but soil resource availability was generally higher in invaded plots. Most herbs benefitted from vegetation removal, indicating release from competition, and growth was not inhibited by the legacy of garlic mustard, either at low garlic mustard densities or after multiple years of removal. Both herb biomass and soil nutrient availability remained high, suggesting the possibility of a positive legacy effect due to nutrient enrichment. Garlic mustard's fertilization effect may be a more important

pathway of impact in woodland herbs than reductions in mycorrhizal associations. These findings are consistent with the “backseat driver” model of invasion in which the invader is not a primary driver of native decline but does contribute to ecosystem change. Restoration plantings may be successful following garlic mustard control, provided other environmental stressors (e.g. deer herbivory) are managed.

Introduction

The nature and mechanisms of plant invasion impacts have important implications for management outcomes and post-invasion restoration of plant communities. There are numerous reports of native plant communities failing to recover after invasion control (Erskine Ogden and Rejmánek 2005, Galatowitsch and Richardson 2005, Bush et al. 2007, Reid et al. 2009, Larson and Larson 2010); four potential explanations for this unsatisfactory outcome include: 1) the impacts of invasion persist at low densities, and insufficient levels of control have been attained to result in community recovery (Norton 2009); 2) the invasive species may negatively affect native species through soil-mediated impacts or other altered ecosystem conditions or processes that persist even after the invader has been removed (Antunes et al. 2008, Jordan et al. 2008, Marchante et al. 2009, Corbin and D'Antonio 2012); 3) the invader may have suppressed native species through non-persistent mechanisms, but due to the prolonged invasion, a native seed bank and propagule sources are no longer present to recolonize the site after invasion (Erskine Ogden and Rejmánek 2005, Vidra et al. 2007, Vilà and Gimeno 2007, Corbin and D'Antonio 2012); or 4) the invader was not actually driving native plant decline, therefore controlling the invader does not result in a positive community response (MacDougall and Turkington 2005, Bauer 2012). These four explanations suggest very different approaches to invasion management and restoration.

Certainly, whether an invasive species is actually driving change and negatively impacting the native plant community is an essential question that should be addressed prior to initiating costly management programs (Didham et al. 2005, MacDougall and Turkington 2005). While “guilty until proven innocent” may be a reasonable approach to

invasion policy and prevention (Ruesink et al. 1995, Simberloff 2005), once an exotic species is well-established, the high costs associated with its control must be weighed against the impacts of its presence and removal (Simberloff and Stiling 1996, McFadyen 1998, Blossey 1999, Blossey et al. 2001b). If invasion is responsible for native species declines but exerts impacts that are only felt in the presence of a living invader, invasion control may be warranted and sufficient for plant community recovery, provided native propagule sources are still present and the seed bank is not dominated by exotic species (Webb et al. 2001, Vilà and Gimeno 2007, Gioria and Osborne 2010, Hughes et al. 2012). When recovery is limited by insufficient propagule sources, this “biotic legacy” of dispersal limitation may be overcome via restoration planting (Daehler and Goergen 2005, Brudvig et al. 2011, Corbin and D'Antonio 2012). Resource competition and, in some cases, altered resource availability (e.g. light) are examples of invasion impacts that may be reversed directly by reducing the cover and density of the invasive species. However, invasion-driven alterations to ecosystem processes and soil chemical, physical and biotic properties may not be reversed in such a straightforward manner (Walker and Smith 1997, Gordon 1998). Such altered site conditions may affect native species growing in the presence of a living invader, and in sites formerly occupied by an invader. Soil legacy effects may therefore continue to inhibit native community recovery after the invader has been controlled, and attempts to assist recovery via restoration planting may have unfavorable results unless sites are returned to pre-invasion conditions (Marchante et al. 2009, Corbin and D'Antonio 2012). Restoration potential and susceptibility to re-invasion are likely a function of both environmental conditions (e.g. climate) and the duration of invasion legacy effects, which may dissipate relatively rapidly or persist indefinitely without intervention (Bates et al. 2000, Blumenthal et al. 2003, Marchante et al. 2009, Pierce and Reich 2010). Soil-mediated impacts and competitive pressures may also persist at low invasion densities, continuing to influence community composition despite otherwise-effective invasion control (Norton 2009). Invasion impacts may not necessarily decline linearly with density reduction, particularly if critical thresholds are crossed that push the system into a resilient “alternative state”; reductions required to sufficiently minimize impacts may not be clear (Levine et al. 2003, Suding et al. 2004,

Richardson et al. 2007, Norton 2009, Suding and Hobbs 2009). This is particularly important because complete eradication of an established invader is only rarely feasible or attainable (Norton 2009, Simberloff 2009).

Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] is an invasive perennial herb that spreads rapidly and appears to impact North American forest and woodland communities via both competitive effects and altered ecosystem conditions (Nuzzo 1999, Scott 2000, Meekins and McCarthy 2002, Evans and Landis 2007, Rodgers et al. 2008a). Garlic mustard possesses many traits that suggest it would be a strong competitor, such as high reproductive output (Anderson et al. 1996), plastic responses to light (Dhillion and Anderson 1999), and flexible nitrogen acquisition strategies (Hewins and Hyatt 2010), and certainly the high densities achieved by garlic mustard suggest competition and shading as a likely mechanism of impact. However, although this hypothesis is supported by some competition experiments (Meekins and McCarthy 1999, Cipollini and Enright 2009), others show native plants to be either unaffected by garlic mustard's competitive pressure (Scott 2000, Wixted 2009), or even capable of outcompeting garlic mustard (Meekins and McCarthy 1999, Murphy 2005). Additionally, garlic mustard has been found to have soil-mediated impacts, altering both soil chemistry and biota in invaded systems (Vaughn and Berhow 1999, Roberts and Anderson 2001, Cipollini 2002, Stinson et al. 2006, Burke 2008, Callaway et al. 2008, Rodgers et al. 2008b, Wolfe et al. 2008). Garlic mustard appears to increase soil pH and nutrient availability (Rodgers et al. 2008b), though the persistence and implications of this fertilization effect for native plant communities have not been tested. More research attention has focused on garlic mustard's potential impact on native plants and mycorrhizal associations via "novel weapons"—glucosinolates (i.e. sinigrin) and secondary compounds with allelopathic and antifungal properties (Roberts and Anderson 2001, Stinson et al. 2006, Callaway et al. 2008, Anderson et al. 2010, Lankau 2010, Cantor et al. 2011, Lankau 2011). In both field and greenhouse studies, tree seedlings growing in the presence of garlic mustard and in soils collected from garlic mustard infestations have exhibited lower biomass and lower root colonization by arbuscular

mycorrhizal fungi (AMF) than tree seedlings in non-invaded areas, or in soils with no history of garlic mustard invasion, suggesting the possibility of a soil-mediated legacy effect (Stinson et al. 2006, Barto et al. 2011).

Although garlic mustard's soil-mediated impacts on tree seedlings have been demonstrated, the extent to which herbaceous woodland plants are affected by garlic mustard's multiple pathways of impact and its removal is less clear. Competition studies suggest that herbs differ in their sensitivity to garlic mustard densities, but several herb species appear to be competitive against the invader (Meekins and McCarthy 1999, Murphy 2005, Cipollini and Enright 2009, Bauer et al. 2010, Hahn and Dornbush 2012). Reported responses of native plants to garlic mustard removal also vary; while some have found an increase in native plant cover following removal (Anderson et al. 2010), most garlic mustard removal studies have not found strong evidence of competitive release (Carlson and Gorchoff 2004, Hochstedler et al. 2007, Bauer et al. 2010), particularly in the case of perennial herbs (McCarthy 1997, Stinson et al. 2007, Herold et al. 2011). This lack of response could be a result of dispersal limitation, which has been documented in North American woodland herbs (Brudvig et al. 2011), but there is also evidence that herb recovery following garlic mustard removal may be delayed because of insufficient mycorrhizal associations in formerly invaded sites (Anderson et al. 2010, Herold et al. 2011). The majority of woodland herbs tested are mycorrhizal (McDougall and Liebtag 1928, Brundrett and Kendrick 1988, Berliner and Torrey 1989, DeMars 1996, Whigham 2004) and are therefore potentially vulnerable to garlic mustard's soil-mediated impacts. Callaway et al. (2008) found that garlic mustard extracts strongly inhibited mycorrhizal colonization of North American herbaceous plants, resulting in reduced seedling emergence, survival and growth, but other studies have reported no reduction in AMF colonization (Burke 2008), or minimal impact on the colonized herbs (Stinson et al. 2006, Koch et al. 2011). Thus, with regard to woodland herbs, it is still unclear whether garlic mustard is, in fact, causing harm, either through competition or ecosystem alterations, and whether its impacts persist following removal.

Native herbs play an important functional role in woodland ecosystems, and reductions in their abundance, diversity and composition may further impact a variety of ecological processes, such as nutrient cycling, microbial activity, and successional trajectories (Muller 2003, Nilsson and Wardle 2005, Gilliam 2007), as well as social and cultural value of woodlands (Chapin III et al. 2000). Declines in herb diversity and abundance have been widely observed (Brewer 1980, Robinson et al. 1994, Rooney et al. 2004, Rooney and Rogers 2011), and a variety of anthropogenic and ecological stressors have been implicated in their loss, including over-harvesting (Jolls 2003); logging (Duffy and Meier 1992, Meier et al. 1995, Small and McCarthy 2002), agriculture (Singleton et al. 2001, Flinn and Vellend 2005), urbanization (Drayton and Primack 1996); herbivory pressure from white-tailed deer (*Odocoileus virginianus* Zimmerman) (Rooney and Waller 2003, Côté et al. 2004, Webster et al. 2005, Wiegmann and Waller 2006) and slugs (Hahn et al. 2011); invasive plants (Woods 1993, Gould and Gorchoff 2000, Collier et al. 2002, Frappier et al. 2003, Greene and Blossey 2011) and earthworms (Bohlen et al. 2004, Frelich et al. 2006, Nuzzo et al. 2009). Inverse correlations between native species and garlic mustard have been cited as evidence that garlic mustard may also be contributing to native herb decline (Nuzzo 1991, Van Riper et al. 2010). Placing the relative impacts of garlic mustard into the broader context of woodland degradation will be important for prioritizing management efforts, particularly given the expense and effort of invasive species control and woodland restoration.

If garlic mustard is driving declines in native herbs, invasion control will be a critical component of woodland restoration. Garlic mustard's potential threat to woodland plant communities and sustainability of North American forests has motivated a biocontrol research program, as conventional control methods are often prohibitively labor-intensive and inadequate once garlic mustard is established (Nuzzo 1991, Blossey et al. 2001a, Gerber et al. 2009, Moser et al. 2009). The interest in biocontrol as a means to manage garlic mustard highlights the need to better understand the nature and mechanisms of garlic mustard's impacts. Successful biocontrol does not eradicate the invader, but reduces the density until an equilibrium is reached with the population of the biocontrol

agent (Simberloff et al. 2005). If native herbs are only negatively impacted by the presence of living garlic mustard, biocontrol may be sufficient for herb recovery, provided that low-enough population densities of garlic mustard are achieved to minimize its impacts. However, if garlic mustard's soil-mediated effects persist following garlic mustard removal, then both biocontrol and conventional control methods may be inadequate for regeneration of native species. Restoration strategies would need to take this legacy effect into account, either by increasing rates of seed/plant addition to compensate for expected losses (a more expensive approach); waiting to plant herbs until the legacy effect subsides (a more cost-effective approach, but one that may make woodlands vulnerable to reinvasion), or by attempting to actively restore altered soil conditions and AMF communities—an approach currently limited by substantial knowledge gaps (Hart and Trevors 2005).

In this study, I investigated the effects of garlic mustard and its removal on native woodland herbs in order to inform garlic mustard control and woodland restoration strategies. Woodland herbs were planted into invaded and non-invaded field plots subjected to various vegetation removal treatments. Garlic mustard's impacts on herbs were assessed by measuring above-ground biomass of herbs two and three years after planting; potential mechanisms of impact were investigated by assessing AMF root colonization of the planted herbs and resource availability (light, soil moisture and nutrients) within the plots. Lower light penetration in invaded plots would suggest that garlic mustard's impacts are mediated through above-ground processes (e.g. shading and competition for light) that depend on the presence of garlic mustard, whereas lower AMF colonization or altered soil chemistry in invaded plots would indicate soil-mediated effects that might persist following garlic mustard removal.

I hypothesized that if garlic mustard negatively impacts herbs, either through competition or soil-mediated pathways, then planted herb biomass would be lower in invaded plots compared to non-invaded plots. To determine whether these impacts depend on the presence of live garlic mustard or persist as a soil legacy effect, I compared herb biomass responses to vegetation removal in invaded and non-invaded

plots. I hypothesized that garlic mustard would have a soil legacy effect that persists even in the absence of live garlic mustard neighbors; therefore I expected to observe an invasion by removal interaction, such that the benefits of vegetation removal would be lower in invaded plots relative to non-invaded plots (Fig. 1a). To determine if low densities of garlic mustard had negative effects on native herbs, I compared biomass of herbs planted into invaded plots subjected to either full, partial or no-removal treatments; if garlic mustard's negative impacts persist at low densities, biomass of herbs planted into partial-removal plots would be more similar to herbs in no-removal plots than to herbs in full-removal plots (Fig. 1b). Finally, I further examined garlic mustard's potential legacy effect by comparing the biomass of herbs planted into invaded plots in which garlic mustard was either left undisturbed or had been removed for one or two seasons prior to planting. If garlic mustard had a legacy effect, I expected to see little difference in herb biomass across treatments, whereas a significant difference between removal duration treatments would suggest a short-term legacy effect (Fig. 1c).

This study differs from previous garlic mustard removal experiments in two ways. Firstly, it includes native vegetation presence and removal control plots, which allow me to evaluate whether garlic mustard uniquely impacts the study species in a manner that is different from native vegetation, while accounting for the disturbance effects associated with removal. Secondly, previous removal studies typically test the response of native plants that either disperse into the site after colonization, which may be a very limited set of species, or resident plants that already occur in the invaded site, which may be biased in favor of species that are relatively tolerant of garlic mustard. By planting herbs into invaded and non-invaded field plots, I separate the impacts of garlic mustard from dispersal limitation; introduce species that may differ in their sensitivity or tolerance to garlic mustard's impacts; and explore the outcomes of active restoration versus passive recovery of woodland herbs following garlic mustard removal.

Methods

Study Sites

This study was conducted in dry-mesic oak forests at two sites: Warner Nature Center (WNC), Marine-on-St. Croix, MN and Cottage Grove Ravine Regional Park (CG), Cottage Grove, MN, located 35 km northeast and 25 km southeast of the city of Saint Paul, respectively. At WNC, the forest canopy is dominated by oak (*Quercus alba* L., *Q. rubra* L.), maple (*Acer rubrum* L., *A. negundo* L.), and black cherry (*Prunus serotina* Ehrh.), and the most common understory species include *Rubus* spp. L., *Athyrium filix-femina* (L.) Roth, *Rhamnus cathartica* L. (seedlings), *Circaea lutetiana* L., *Galium aparine* L., *Geum canadense* Jacq. and *Desmodium glutinosum* (Muhl. ex Willd.) Alph. Wood (L. Van Riper, unpublished data). Other ground-layer species frequent in the immediate study area include *Amphicarpaea bracteata* (L.) Fernald, *Thalictrum thalictroides* (L.) Eames & B. Boivin, *Eurybia macrophylla* (L.) Cass., *Geranium maculatum* L., *Parthenocissus* sp. Planch., *Maianthemum racemosum* (L.) Link ssp. *racemosum*, *Stellaria media* (L.) Vill., and *Trientalis borealis* Raf.

Dominant tree species in the CG study area include oaks (*Quercus macrocarpa* Michx., *Q. ellipsoidalis* E.J. Hill, and *Q. rubra*), black cherry (*P. serotina*), hackberry (*Celtis occidentalis* L.), ironwood (*Ostrya virginiana* (Mill.) K. Koch), eastern red cedar (*Juniperus virginiana* L.), basswood (*Tilia americana* L.) and paper birch (*Betula papyrifera* Marsh.), with buckthorn (*R. cathartica*), black current (*Ribes nigrum* L.), common prickly ash (*Zanthoxylum americanum* Mill.), nannyberry (*Viburnum lentago* L.), red elderberry (*Sambucus racemosa* L. var. *racemosa*), and prickly gooseberry (*Ribes cynosbati* L.) common in the shrub layer, and an understory of *Osmorhiza claytonii* (Michx.) C.B. Clarke, *Geum aleppicum* Jacq., *Hackelia virginiana* (L.) I.M. Johnst., *Maianthemum canadense* Desf. , *Ageratina altissima* (L.) King & H. Rob., *C. lutetiana*, *G. aparine*, and *O. virginiana* seedlings (Brauer & Associates, LTD 2007, L. Van Riper, unpublished data, and pers. obs.). Taxonomy follows USDA, NRCS (2011).

Like many urban and suburban North American woodlands, both sites have abundant populations of white-tailed deer (*Odocoileus virginianus*) and are heavily invaded by buckthorn, garlic mustard, and other invasive plants. European earthworms and slugs are common in both sites, but appeared to be more abundant at WNC (pers. obs.). Standing litter levels are low, and bare ground is common (Van Riper et al. (2010) and personal observation). Soils are primarily Kingsley sandy loam in the WNC study area, and Mahtomedi Loamy Sand in the CG study area (Soil Survey Staff 2010).

Impacts of Garlic Mustard and its Removal on Restored Herbs

To investigate the impacts of garlic mustard on woodland herb restoration, plots were established within a dense garlic mustard infestation and a nearby non-invaded area at each of the two study sites. Plots were subjected to annual vegetation removal treatments and planted with native herbs. The study was replicated in two planting years (2006 and 2007), and aboveground biomass was harvested in 2009 when plants were three and two years old, respectively.

Study Species

Twelve species of perennial herbs native to Minnesota's oak woodlands, representing nine plant families and a range of life history characteristics, were selected for these studies (Table 1). One species (*Allium tricoccum*) is a spring ephemeral, while the others are summer-dominant herbs. Among the summer herbs, flowering time ranged from May-June (e.g. *Geranium maculatum*, *Phlox divaricata*) to August-September (*Solidago flexicaulis* and *Symphyotrichum cordifolium*). Species selected also included a range of expected mycorrhizal strategies (McDougall and Liebttag 1928, Boerner 1986, Newman and Reddell 1987, Brundrett and Kendrick 1988, DeMars 1996, Miller et al. 1999); some species, such as *Hydrophyllum virginianum*, were expected to be non-mycorrhizal, whereas others (e.g. *Maianthemum racemosum*) have been found to be good mycorrhizal hosts.

Plant materials (plugs or bare rootstock, as available) were purchased from three local native plant nurseries (Landscape Alternatives, Shafer, MN; Prairie Moon Nursery, Winona, MN; Prairie Restorations, Inc., Princeton, MN). One species, *Maianthemum racemosum*, was planted both as plugs and bare rootstock in the first year of the study to compare results by plant form. Bare rootstock for *Maianthemum racemosum* and *Actaea rubra* were not available in the second planting year and were replaced with *Mainthemum stellata* and *Solidago flexicaulis* (Table 1).

Experimental Design

In each of the two study sites, 96 semi-permanent plots were established in a dense garlic mustard infestation, and 48 plots were established in a nearby non-invaded area with a cover of primarily native species. The plots were 1 m² and arranged in a randomized grid, with transects placed 8 meters apart. At CG, the native vegetation in the non-invaded area was too sparse to use the grid formation, as numerous plots would have been located on bare ground, which would not provide a reasonable comparison to invaded plots. Plot locations were therefore selected randomly from 66 vegetated patches (>1 m²) with a minimum of 3 species and 50% cover.

One half of the plots (48 invaded; 24 non-invaded per site) were randomly assigned to the restoration experiments and treatments described herein (144 plots total), and the remaining plots were used for a seed addition study (data not presented). Plots were randomly assigned to vegetation removal treatments and planting years, such that there were 6 replicates of each treatment combination (site by invasion by removal by planting year).

Three levels of removal treatments were applied to invaded plots: full removal, partial removal, and no removal (Figure 2). Only full and no removal treatments were applied to non-invaded plots. Full removal involved hand-pulling all vegetation (including roots) from the study plot and a ½ meter buffer around the plot. In partial removal plots, two adult garlic mustard plants and 5 rosettes were left in the plot, simulating the low

densities that might be achieved via successful biocontrol. The removed vegetation was bagged, dried and weighed. All vegetation was left undisturbed within the no-removal plots and surrounding buffer area. Removal treatments were reapplied annually (May – June), as garlic mustard in particular heavily re-seeded into the experimental plots from the surrounding vegetation.

Six plots from each site by invasion by removal treatment combination were planted in year 1 (2006). Full and no-removal plots in invaded and non-invaded areas were used to compare the effects of garlic mustard and its removal on planted woodland herbs, relative to the effects of native vegetation. The partial removal plots were compared to (invaded) full and no-removal plots to assess whether garlic mustard impacts on native herbs would persist at low densities.

These experiments were replicated in year 2 (2007); again, six plots from each site by invasion treatment were assigned to full, partial or no-removal treatments and were then planted. In addition, plots that were subjected to removal treatments but left unplanted in year 1 were planted in year 2 following a repeated removal treatment. The performance of herbs planted into these plots were compared to that of herbs planted into year-2 removal plots to determine whether the impacts of garlic mustard persisted after two seasons of removal prior to planting (i.e. a legacy effect).

Planting

Woodland herbs were fall-planted (Sept. – Oct.), approximately four months following vegetation removal treatments. Ten species were planted into each plot in year 1, and 11 species were planted in year 2 (Table 1). Two or three individuals per species were planted into each plot in a randomized grid (Year 1: 29 total per plot; Year 2: 30 total per 1 m² plot). Plugs from a given six-pack were separated so that plots did not receive multiple plants from the same six-pack.

The weather was unseasonably warm and dry in autumn of Year 1, so all planted herbs were lightly irrigated via back-pack sprayers immediately following planting. Precipitation was abundant in autumn of Year 2, and irrigation was not necessary. In early December of Year 1, I mulched plots with a light layer of litter from the surrounding area to protect plants from frost heave. The mulch layer was removed in early April the following spring. In Year 2, I did not mulch plots, but I did attempt to minimize disturbance to the existing litter layer while planting. All plots were fenced with 3-foot (0.91 m) tall, 2 by 3 inch (5.1 by 7.6 cm)-cell galvanized steel yard fencing to protect herbs from herbivory by deer. Damaged plot stakes and fencing (due to storms, tree falls and other natural causes) were replaced as necessary throughout the 3-year study.

Assessment of Environmental Variables and Resource Availability

Light levels, and soil texture, moisture, pH, and nutrient availability were measured in order to characterize the study sites and account for differences between sites and treatments that might influence plant responses (i.e. by including as covariates in the analyses of biomass responses), as well as to investigate how garlic mustard and its removal affect resource availability. Light and soil moisture were measured in all study plots. Soil pH and nutrients were measured in full- and no-removal treatments of year 2-planted plots only. Soil texture was measured in a subsample of plots in the invaded and non-invaded areas at each site.

Light Availability

As a limiting resource in the forest understory (Tremblay and Larocque 2001, Neufeld et al. 2003, Whigham 2004), light availability can strongly influence plant productivity (Ellison and Houston 1958). The amount of light that can penetrate to the herb layer is affected by the tree canopy openness. To account for the effect of light on native herb growth, I recorded diffuse non-interceptance (DIFN) light levels using LAI-2000 plant canopy analyzers (Li-Cor Inc., Lincoln, NE, USA) in 2008 and 2009.

Measurements were taken in spring (April) and mid-summer (July) to capture light availability both before and after tree canopy closure. Light was measured at dawn, dusk or in otherwise overcast conditions to capture ambient light levels with minimal influence of sun angle. To calculate the percentage of light penetration through the overstory canopy, below-canopy measurements obtained one meter above each plot were divided by above-canopy measurements obtained from a unit placed in a nearby open field, monitoring sky conditions every 15 seconds. I also measured light levels at the forest floor (~5 cm height) in the same manner. The purpose of above-plot measurements was to characterize the study sites and account for variability in light availability across treatments. The forest-floor light measurements were recorded to investigate how garlic mustard and its removal affect light availability to restored herbs relative to native (non-invaded) vegetation.

Soil Texture, Moisture and pH

Soil texture influences water holding capacity and nutrient availability. To ensure that potential differences in soil texture and moisture were not driving plant responses to invasion treatments, I analyzed soil texture from a random subsample of plots within each invasion treatment at each site. In 2007, I collected soil (1-10 cm depth) from 10 plots in the non-invaded area and 12 plots in the invaded areas at each study site. Soils were air dried, ground and sieved, then analyzed for texture via hydrometer method (Day 1965). Soil moisture was also measured directly in all study plots in mid-September, 2008 using a TDR (time domain reflectometry) device (to 10 cm depth).

To analyze soil pH, I collected soil samples (10 cm depth) from Year 2-planted plots in early September, 2007. The soil was air-dried and mixed with water in a 1:2 ratio. The pH of the resulting slurry was measured with a Beckman 10 pH meter (Beckman Coulter, Inc., Brea, CA, USA). Garlic mustard has been found to increase soil pH, and this is a potential mechanism by which garlic mustard may increase soil phosphorus availability (Rodgers et al. 2008b).

Nutrient Availability

I measured the availability of phosphorus (P), nitrate (NO_3^-) and ammonium (NH_4^+) to determine whether these nutrients levels were higher in invaded soils, as has been previously reported (Rodgers et al. 2008b), to explore potential responses to vegetation removal treatments, and to account for their influence on herb biomass production. In full- and no-removal Year 2-planted plots, I buried nylon bags containing 15 ml of acid-washed anion-exchange resins at approximately 10 cm depth. In 2007, a single resin bag was placed in each plot for two time periods: May – July (during initial vegetation removal treatments) and August – September (following removal treatments). Because numerous resin bags were lost (presumably disturbed by deer and burrowing mammals), the following year, two resin bags were placed per plot for a single time period (May – August) to ensure at least one measurement per plot.

Resins were kept frozen until extraction. To extract the nutrients, resin bags were thawed and rinsed in Nanopure water, air-dried for one week and weighed. The dried resins were placed in 30-ml syringes with a glass microfiber filter and rinsed with 100 ml of 2M NaCl in 0.1M HCl. The extract was transferred to plastic culture tubes and frozen. I analyzed soluble reactive phosphorus (SRP) using the methods of Strickland and Parsons (1972). Nitrogen was analyzed colorimetrically on an Alpkem RFA 300 Autoanalyzer at the Soil Testing Laboratory of the University of Minnesota, St. Paul, MN (<http://soiltest.cfans.umn.edu/>). For all nitrogen and 2007 phosphorus measurements, a single resin sample was analyzed for each plot and measurement period. However, two phosphorus resin samples were analyzed per plot for the 2008 measurements; the results were averaged per plot for statistical analysis.

Statistical Analysis of Environmental Variables and Resource Availability

Differences in above-plot light availability, soil texture, moisture, pH, and nutrient availability by site and treatments were analyzed individually with ANOVA (proc glm;

SAS 9.2). All response variables, except soil texture variables were natural log-transformed to improve normality.

For soil texture, differences in percent sand, silt and clay were analyzed by site and invasion area. I analyzed soil moisture and above-plot light availability by site, invasion and removal treatments separately for each planting year. Soil pH and nutrients were analyzed by site, invasion and removal (full- and no-removal treatments) in Year 2-planted plots only. Nutrient data was analyzed separately for each measured time period (early and late summer 2007, and full summer 2008).

I also analyzed the response of soil nitrogen availability to the duration of garlic mustard removal treatments. I compared the availability of soil N in late summer 2007 between plots from which garlic mustard had been removed for one or two seasons prior to the measurement period, and I compared soil N availability in summer 2008 between plots from which garlic mustard had been removed for two or three seasons prior to the measurement period.

I analyzed forest floor light availability by invasion and removal treatments with above-plot light included as a covariate. ANCOVA analyses were run separately for each site, because the range of values of above-plot light levels did not overlap on the two sites.

I analyzed relationships between all environmental variables with simple linear correlations (proc corr; SAS 9.2) to determine whether potential covariates in the plant biomass analyses were strongly correlated. Soil nutrient measurements and pH were analyzed with nonmetric multidimensional scaling (NMS; PC-Ord 5.32) for each of the 2007-planted analyses (excluding degree of removal), producing a synthetic axis that was included as a covariate. In each case, the axis was defined primarily by the three NO_3^- measurements; the axis generated for the invasion by removal analysis accounted for 89% of the variation in the data, and the axis for the duration of invasion analysis

accounted for 82% of the variation in the data. Plots that were missing pH or nutrient measurements were excluded from the NMS and ANCOVA analyses.

Assessment of Herb Biomass Responses to Invasion and Removal Treatments

I recorded the presence and size (percent cover) of planted herbs monthly in summer 2007 (June, July and August), and in June and August of 2008. Presence data was used to monitor plant survival and distinguish between initial overwintering mortality in the first planting year and subsequent mortality. Percent cover of individual planted herbs was estimated as a proxy for biomass in the first two years of the study (data not presented herein) and used to select timing of biomass harvest. I regularly recorded observations of herbivory, flowering and fruiting of individual plants. Storm damage to plots and individual plants was also recorded. Falling trees were common in both sites, and in June 2008, a tornado occurred at the WNC field site causing substantial damage. Small fallen trees and limbs were removed from plots manually, and storm-damaged plots were included in the study unless they were completely covered by a very large tree fall and were no longer accessible.

In autumn 2008 and summer 2009, I collected aboveground biomass from all planted herbs, including both 2- and 3-year old plants (Year 2- and Year 1-planted, respectively). Each species was harvested in the month of peak biomass, as determined by percent cover measurements in the preceding years (Table 1). Harvested plants were individually bagged, dried for 7-10 days at 60°C, and weighed.

Statistical Analysis of Herb Biomass

Six species in year 1-planted plots and eight species in year 2-planted plots had sufficient survival at both study sites to be included in a multi-species analysis; these species are hereafter referred to as “WNC/CG species”. Three species in each planting year (*Allium tricoccum*, *Symphytotrichum cordifolium* and *Phlox divaricata* in Year 1; and *A. tricoccum*, *S. cordifolium*, and *Osmorhiza claytonii* in Year 2) had very high mortality

at WNC and could not be analyzed for that site. These species, hereafter referred to as “CG-only species” were analyzed separately from the other species at CG, so that site differences could be more clearly inferred for the WNC/CG species. Year 1-planted *O. claytonii* had poor survival at both sites and was excluded from the analyses. Plants that suffered from overwintering mortality in the initial planting year were excluded from the analyses, but subsequent mortality was entered as a biomass of 0 g.

Herb biomass was analyzed with multi-species mixed models with plot as a random effect, species, invasion and removal treatments as fixed effects; and above-plot light as a covariate (ANCOVA; proc mixed; SAS 9.2). Summer (post canopy closure) 2009 light data was used for the covariate, as it was the most complete data set for each site. Soil moisture content was not included in the analyses, as it was positively correlated with above-plot light levels ($r^2 = 0.75$; $p < 0.0001$.) The analyses of full- and no-removal Year 2-planted plots were also run with the synthetic nutrient axis included as a covariate, to compare the apparent effects of garlic mustard both with and without taking soil nutrient availability into account. I ran separate analyses for two and three year old plants; sites were also analyzed separately because the range of values of the light covariate did not overlap between the two sites. In all analyses, herb biomass was natural log transformed to improve normality.

Three sets of analyses were completed: 1) Analysis of the effects of living garlic mustard and its removal relative to that of native vegetation at each of two sites and in two planting years; invasion and removal treatments each had two levels: invaded or non-invaded; full or no removal; 2) Analysis of the persistence of garlic mustard’s impacts at reduced densities at two sites and in two planting years; in this analysis, the removal treatment had three levels: full, partial and no removal; 3) Analysis of duration of garlic mustard removal prior to planting to determine potential legacy effects at two sites, in year 2-planted plots only; in this analysis, the removal treatment had three levels: two years, one year, and no-removal.

AMF Colonization of Herb Roots in Invaded and Non-Invaded Areas

Roots were collected from invaded and non-invaded (no-removal) plots at each study site to assess differences in AMF colonization as a result of garlic mustard invasion. While harvesting plant biomass, I collected root samples from one randomly selected plant per species per plot. Root samples were kept in a cooler in the field, washed to remove soil particles, and frozen for storage. In preparation for AMF visualization, thawed roots were cleared by autoclaving in KOH, rinsed with distilled water, acidified with HCl, and then stained in aniline blue (Grace and Stribley 1991). Roots of three species (*Actaea rubra*, *Geranium maculatum*, and *Mitella diphylla*) were particularly dark or opaque; to aid in clearing the roots, I soaked them in bleach (Sodium hypochlorite) for 1 min. and then rinsed thoroughly prior to staining.

I arranged a representative sub-sample of fine roots on a slide and viewed the roots at 200x magnification with a bright field microscope. Colonization was quantified by systematically viewing the slide and recording the presence or absence of AMF structures in each field of view (McGonigle et al. 1990). I recorded AMF as present if there were vesicles, arbuscules, or hyphae (Figure 3) visible within the root (Smith and Read 2008). Spores and “loose” hyphae (not penetrating the root) were not considered evidence of AMF colonization. I calculated the percentage of root colonized by AMF by dividing the presence by the total number of views and multiplying by 100.

Additionally, to determine whether the nursery-grown herbs were already colonized by AMF prior to planting in experimental plots, I collected root samples from five individuals per species (Year 2-planted plots only). I harvested the root samples on the first day of field planting, and then quantified AMF following the same methods described previously.

Statistical Analysis of AMF Colonization of Herb Roots

Six species of three-year old herbs (planted in year 1) had sufficient survival at both study sites to be evaluated for a two-site, multi-species analysis of AMF colonization

(Table 1); of these only four species had evidence of AMF colonization in roots and were analyzed statistically. Bare root and plug-planted *Maianthemum racemosum* were combined for this analysis. Three species had poor survival at WNC, and insufficient root samples were available from this site for analysis. Roots of these species were analyzed from CG herbs only, but both two- and three-year-old plants were included in the multi-species analysis (Table 1). *Solidago flexicaulis* also had poor survival at WNC, but was only planted in 2007; roots from two-year *S. flexicaulis* were therefore analyzed separately from the other CG-only species. *Osmorhiza claytonii* and *Maianthemum stellatum* had high mortality at both sites and could not be analyzed.

Percent colonization of roots by AMF was analyzed with a mixed-effects model (proc mixed; SAS 9.2) with plot as a random effect, and site (or planting year, for the species collected only at CG), species and invasion as fixed effects. *Solidago flexicaulis* was analyzed separately with only invasion as a factor (proc glm; SAS 9.2) Percent colonization data was arcsine-square root transformed to improve normality.

Assessment of AMF in Resident Woodland Herbs

To assess the level of AMF colonization in resident (non-planted) woodland herbs, in late-summer 2006, I collected root samples from four common native species (Table 1) that could be found growing within a dense garlic mustard infestation and a nearby non-invaded area at WNC. Samples were collected from 12 mature plants in each area (invaded and non-invaded) for a total of 24 root samples per species. (Only 14 root samples of *Geranium maculatum*, six from the invaded area and eight from the non-invaded area, could be analyzed, due to problems with the staining procedure.) I also collected root samples from 12 adult (second-year) garlic mustard plants to verify the non-mycorrhizal status of this species. Plants were selected haphazardly (non-systematically), but were at least 10 m apart. Roots were processed and AMF quantified following the same methods described for the assessment of AMF in resident plants.

I analyzed percent root colonization by AMF in the four native species with a two-way ANOVA model (proc glm; SAS 9.2), with species, invasion and their interaction included as factors. Garlic mustard root samples were not statistically analyzed, as there were no experimental treatments for these roots, and no evidence of AMF colonization was observed.

Results

Environmental Variables and Resource Availability by Site, Invasion & Removal Treatments

Site Differences

The two study sites differed significantly in most abiotic attributes measured (Table 2). Plots at WNC had significantly higher above-plot light availability and soil moisture than the CG plots, which were located on north-facing slopes. Soils at WNC were more acidic and ranged from loamy sand to sandy loam, with significantly higher silt and clay and lower sand content than CG, where soils were sandier. Availability of NO_3^- and NH_3^+ was generally higher in WNC study plots, while P availability was similarly high at both sites.

Effects of Invasion and Vegetation Removal on Light Availability

Garlic mustard invasion did not appear to affect light availability to restored herbs relative to the effects of non-invaded native vegetation. Forest floor light availability did not differ between invaded and non-invaded plots at either site, but did increase in response to vegetation removal treatments (Fig. 4). At CG, there was a significant above-plot light by removal treatment interaction ($p < 0.0001$): forest-floor light availability increased along with increasing above-plot light levels in removal plots, but not in no-

removal plots. At WNC, forest floor light availability was increased by removal treatments, although not significantly ($p = 0.07$).

Differences in soil chemistry and resource availability by invasion and removal treatments

Soil texture and pH did not differ between garlic mustard-invaded and non-invaded plots at either site, but invaded plots did have higher soil moisture and nutrient availability than non-invaded plots (Table 2). Soil moisture was significantly higher in invaded plots than non-invaded plots when analyzed across planting years ($p = 0.02$), and within year 1-planted plots ($p = 0.03$), but differences were not significant in year 2-planted plots. Neither soil moisture nor pH was affected by vegetation removal treatments (soil texture was not analyzed by removal treatment).

Invaded plots had consistently higher P availability than non-invaded plots in all three measurement periods (Table 2; 2007: $p = 0.007$), although the magnitude of difference was greater at CG than at WNC (site by invasion by removal interaction $p = 0.05$ in 2008). Removal treatments had little impact on soil P availability. Although P was higher in no-removal plots in both 2007 measurements, the effects were not significant, and in 2008 effects of removal varied by site and invasion.

Both soil NO_3^- and NH_3^+ availability were generally higher in invaded plots than in non-invaded plots, although, like P, the differences were much greater at CG than at WNC (Table 2; Fig. 5 a, b). Effects of invasion on NO_3^- differed by both site and removal treatment in early summer 2007 (interaction $p = 0.03$), and by site in late summer 2007 (interaction $p = 0.03$; Fig. 5a); while invaded plots had higher NO_3^- at both sites, the magnitude of difference was greater at CG ($p < 0.0001$) than at WNC ($p = 0.07$). In 2008, after two seasons of garlic mustard removal, NO_3^- was significantly higher in invaded plots ($p = 0.0002$) and in removal plots ($p = 0.03$), but the invasion by removal interaction was not significant ($p = 0.09$; Fig. 5b). Similarly, effects of invasion on NH_3^+

differed by site or were insignificant in 2007, whereas in 2008, the invasion main effect was significant without treatment interactions.

Elevated levels of soil nitrogen appeared to persist following garlic mustard removal. In the analysis of vegetation removal effects in invaded and non-invaded plots, vegetation removal had variable impacts on both NO_3^- and NH_3^+ in the initial year of removal (2007), but in 2008, after two seasons of removal treatments, NO_3^- availability was significantly higher in full removal plots than in no-removal plots ($p = 0.03$). Removal treatments appeared to have a greater effect on NO_3^- availability in invaded plots than in non-invaded plots, but the invasion by removal interaction was not significant ($p = 0.09$; Fig. 5b). The effect of removal on NH_3^+ was not significant in 2008, although as with NO_3^- , the effects of removal were marginally greater in invaded plots (invasion by removal $p = 0.08$).

The analysis of soil nitrogen in response to the duration of garlic mustard removal treatments also suggested a persistent fertilization effect. Although the removal treatment effect was only marginally significant, soil NO_3^- appeared to be elevated in removal plots relative to no-removal plots at both sites in late summer 2007 ($p = 0.06$; Fig. 5c) and in summer 2008 ($p = 0.07$; Fig. 5d), with no evidence of a decline in NO_3^- after multiple years of sustained garlic mustard removal. In both measurement periods, the plots with prolonged vegetation removal (two years of garlic mustard removal in 2007, and three years of removal in 2008) had comparable or higher NO_3^- as plots from which garlic mustard had been removed more recently.

Effects of Invasion and Vegetation Removal on Restored Herb Biomass

Overall, garlic mustard had a facilitative—not inhibitory— effect on woodland herb restoration, as most planted herb species had higher biomass in invaded plots than in non-invaded plots. Garlic mustard's positive effect on herb biomass appeared to be explained in part by nutrient effects and was not contingent on light levels. Vegetation removal treatments generally resulted in higher herb biomass, with stronger effects in invaded

plots than in non-invaded plots. Biomass of most herb species was higher at the more resource-rich WNC site than at CG, and there were differences in herb response to both invasion and removal by study site and species.

Cottage Grove

Garlic mustard invasion generally had a positive effect on biomass of three-year-old herbs at CG, although the effects of invasion differed by species, removal and, in CG-only species, by above-plot light levels. In WNC/CG herbs there was a significant invasion by species by removal interaction ($p = 0.05$; Fig. 6a). Four of these species had higher biomass overall in invaded plots; while this pattern was consistent across removal treatments for *Actaea rubra*, *Mitella diphylla*, and *Thalictrum dioicum*, invasion effects on *Hydrophyllum virginianum* depended on removal treatment. Within no-removal treatments, *H. virginianum* had significantly higher biomass in invaded plots relative to non-invaded plots ($p = 0.009$), but there was no difference between invaded and non-invaded removal plots. In non-invaded plots, vegetation removal had minimal effect on *H. virginianum*, but garlic mustard removal negatively impacted biomass of this species ($p = 0.009$). Within other WNC/CG species, the effects of vegetation removal were variable and non-significant. The above-plot light covariate was not significant in the analysis of WNC/CG herbs, however, in the analysis of CG-only species, both invasion by species and removal by species interactions depended on the light level ($p = 0.0008$ and 0.04 , respectively; Fig. 6c). *Phlox divaricata* biomass was consistently higher in invaded plots, regardless of light level, whereas *Allium tricoccum* biomass was consistently lower in invaded plots. *Symphytotrichum cordifolium* had lower biomass in invaded plots at low light levels but higher biomass in invaded plots at medium and high light levels ($p = 0.05$ and 0.01 , respectively).

Two-year-old herbs at CG had similar responses to invasion and removal as three-year-old herbs. Biomass of WNC/CG herbs was generally higher in invaded plots compared to non-invaded plots, but the effects of invasion varied by species ($p < 0.0001$; Fig. 7a). Five of these species had significantly higher biomass in invaded plots; the

invasion effect was non-significant in the remaining three species. Most WNC/CG species benefited from vegetation removal, although removal effects also differed by species ($p = 0.02$). The invasion by removal interaction was nearly significant ($p = 0.06$); the benefits of removal were greater in invaded plots than in non-invaded plots. WNC/CG species responded differently to above-plot light levels (light covariate by species $p = 0.03$), but none of the treatments interacted significantly with light.

Results of two-year-old CG-only herbs differed from three-year-old plants in that the light covariate did not interact significantly with treatments. The effects of garlic mustard invasion on two-year-old CG-only herbs depended on both herb species and vegetation removal treatment ($p = 0.002$; Fig. 8a). All three species had consistently higher biomass in invaded plots, but the magnitude of invasion impact depended on removal treatments in two species. *Osmorhiza claytonii* had significantly higher biomass in invaded plots than non-invaded plots within no-removal treatments ($p = 0.03$), but showed less of an invasion response in removal plots. *Symphyotrichum cordifolium*, however, had a strong positive response to invasion within removal plots ($p < 0.0001$) but no effect within no-removal plots. Garlic mustard removal resulted in a strong positive response by *S. cordifolium* ($p = 0.0005$), while removal of vegetation from non-invaded plots had minimal impact.

A second analysis of two-year-old herbs was run with the synthetic nutrient axis included as a covariate in order to compare the effects of invasion with and without the nutrient pathway accounted for (soil nutrient data was not available for three-year-old herbs). When soil nutrient availability was factored into the analysis, the facilitative effects of invasion on herb biomass at CG appeared to be minimized (Fig. 7b). Overall, biomass was still higher in invaded plots compared to non-invaded plots in both WNC/CG and CG-only species, and the invasion by removal by species interaction was significant for both WNC/CG and CG-only species ($p = 0.04$ and 0.002 , respectively), but within-species, the effects of invasion were no longer significant for any species, with the exception of *Symphyotrichum cordifolium*, which still had significantly higher biomass in invaded plots within the removal treatment ($p < 0.0001$; Fig. 8b). Three

species had significant positive responses to garlic mustard removal (*Geranium maculatum* $p = 0.03$; *S. cordifolium*, $p = 0.0003$; *Thalictrum dioicum* $p = 0.005$), while only one species had a significant positive response to native vegetation removal (*Hydrophyllum virginianum*, $p = 0.004$). WNC/CG species responded differently to both light and nutrient availability ($p = 0.02$ and $p = 0.004$, respectively), and removal effects also depended on nutrients ($p = 0.03$). However, neither light nor nutrients interacted significantly with the invasion treatment. CG-only species were not significantly affected by light or nutrient availability.

Warner Nature Center

As at CG, biomass of three-year-old herbs at WNC was generally higher in invaded areas, but the effect of invasion differed by species ($p = 0.02$; Fig. 6b). In four species, biomass was higher in invaded; although the effect was only significant in *Thalictrum dioicum*; ($p = 0.01$) and nearly significant in *Actaea rubra* ($p = 0.06$). In *T. dioicum*, this positive effect of invasion appeared to be driven by strong responses within no-removal plots. Overall, vegetation removal had a positive effect on herb biomass, but the effects differed by species and above-plot light level ($p = 0.01$). Unlike CG, there was no significant invasion by removal interaction for three-year-old plants at WNC. While site differences could not be statistically evaluated, some species (e.g. *Geranium maculatum* and *Hydrophyllum virginianum*) appeared to respond differently to invasion at WNC than at CG (Fig. 6 a, b).

Two-year-old herbs at WNC differed from both three-year-old herbs at WNC and herbs at CG, in that they were not significantly affected by invasion (Fig. 9a). Although *Hydrophyllum virginianum* and *Thalictrum dioicum* appeared to have lower biomass in invaded plots within no-removal treatments, these effects were not significant. Most species had higher biomass in vegetation removal plots, but the effects of removal differed by species and light levels ($p = 0.01$). Inclusion of the nutrient covariate in the analysis of second-year-herbs at WNC did not affect individual herb biomass responses to invasion as clearly as at CG. The invasion main effect and interactions with vegetation

removal were still insignificant, however there was a significant invasion effect that depended on nutrient level ($p = 0.05$; data not shown). At low nutrient levels, biomass was lower in invaded plots, but at higher nutrient levels, biomass was higher in invaded plots.

Herb Biomass Responses to Degree of Garlic Mustard Removal

The degree of garlic mustard removal (full, partial or no-removal) had little impact on herb biomass. The effects of different degrees of removal were analyzed for both three- and two-year-old herbs at each study site. At the time of harvest, three-year-old plants had experienced three years of garlic mustard removal. Two-year-old plants were growing in plots that had either two or three total years of garlic mustard removal (removal treatments initiated in 2007 and 2006, respectively); separate analyses were run for each. Only species with sufficient survival at both study sites (WNC/CG species) were included in these analyses, and nutrient data was not available for partial removal plots, so it could not be included as a covariate. Across all analyses, herbs generally had higher biomass in removal plots (full or partial) than in no-removal plots, but the effects of garlic mustard removal were only significant in two-year-old plants at WNC.

Cottage Grove

At CG, the degree of garlic mustard removal had no significant effects on herb biomass when analyzed across species, regardless of plant age or number of years of removal (Fig. 10 a, c). Herb biomass was generally higher in full and partial removal plots compared to no-removal plots, but only the species main effect and light covariate were significant ($p < 0.0001$ and 0.03 , respectively).

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In general, three- and two-year old herbs at WNC also responded positively to garlic mustard removal, however they differed in their response to the degree of garlic mustard removal. As at CG, three year old herb biomass was affected by species and light levels

($p < 0.0001$), but the removal treatment effect was not significant. Two-year-old herbs at WNC did, however, respond significantly to removal treatments. Although the removal main effect was not significant, herbs planted into plots with three years of garlic mustard removal had significantly higher biomass in full removal plots than in no removal plots ($p < 0.01$ with Bonferroni correction); the light by species interaction was also significant ($p < 0.0001$). Two-year-old herbs in plots with two years of garlic mustard removal also responded significantly to light levels ($p = 0.0008$), but also had more varied responses to removal. The removal by species interaction was significant ($p = 0.02$); *Solidago flexicaulis* and *Thalictrum dioicum* had significantly higher biomass in full removal plots than in no-removal plots ($p = 0.01$ and 0.0009 , respectively), and nearly significant differences between partial and no-removal plots as well ($p = 0.09$ and 0.07 , respectively). *Geranium maculatum* had significantly higher biomass in partial removal plots than in no removal plots ($p = 0.02$), with full removal plots intermediate. *Hydrophyllum virginianum*, on the other hand, had significantly higher biomass in full removal compared to partial removal, with nearly significant differences between full and no-removal ($p = 0.08$) and partial and no-removal ($p = 0.07$).

Herb Biomass Response to the Duration of Garlic Mustard Removal

Restored herbs at both study sites benefited from garlic mustard removal, and whether garlic mustard had been removed for one or two seasons prior to planting—for a total of three or two years of sustained removal treatments at the time of harvest—had little impact on herb biomass. However, herb responses to the duration of removal treatments differed somewhat between study sites, with nutrient availability affecting the response at CG but not at WNC.

Cottage Grove

At CG, removal duration and species effects both depended on nutrient levels ($p = 0.04$ and 0.006 , respectively). At low nutrient levels, removal effects were insignificant, but at medium and high nutrient levels, biomass was highest in plots with two years of

removal, intermediate in plots with three years of removal, and lowest in no-removal plots. At medium nutrient levels, biomass in two-year removal plots was significantly higher than in no-removal plots ($p = 0.02$; Fig. 11a), and at high nutrient levels both two- and three-year removal plots were significantly higher than no-removal ($p = 0.02$ and 0.03 , respectively). There was also a significant removal by species interaction ($p = 0.05$); individual species differed in whether two or two years of removal resulted in the highest biomass, but except for *Maianthemum racemosum* and *M. stellatum*, species had higher biomass in removal plots than no-removal plots (Fig. 11b). Three species (*Geranium maculatum*, *Hydrophyllum virginianum* and *Thalictrum dioicum*) had significantly higher biomass in two-year removal treatments compared to no-removal ($p = 0.0006$, 0.004 , and < 0.0001 , respectively); *H. virginianum* and *T. dioicum* also had significantly higher biomass in three-year removal plots compared to no-removal plots ($p = 0.04$ and 0.03 , respectively). *Mitella diphylla* had near significant differences between both two-year and three-year removal treatments compared to no-removal treatments ($p = 0.06$ and 0.07 , respectively). Biomass did not differ significantly between two and three years of removal for any species, although the difference was nearly significant in *G. maculatum* ($p = 0.06$). The light covariate was also significant in this analysis ($p = 0.02$), but did not interact with other factors.

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At WNC, both duration of removal and species effects were significant ($p = 0.01$ and < 0.0001 , respectively; Fig. 11c), but unlike at CG, their effects did not depend on nutrient levels. Herb biomass in plots with two or three years of removal was significantly higher than in plots with no removal ($p = 0.03$ and 0.004 , respectively), but they did not differ significantly from each other (Fig. 11a). *Geranium maculatum*, *Hydrophyllum virginianum*, *Solidago flexicaulis* and *Thalictrum dioicum* appeared to have particularly strong responses to garlic mustard removal. The light covariate was also significant ($p = 0.006$), but did not interact with other treatment factors.

Effects of Garlic Mustard Invasion on AMF Root Colonization in Restored Herbs

Prior to planting, AMF colonization rates in nursery plant roots were variable both within and between species (Table 3). *Geranium maculatum*, *Hydrophyllum virginianum*, *Mitella diphylla* and *Solidago flexicaulis* had no evidence of root colonization; while minimal (< 5%) colonization was observed in *Maianthemum racemosum*, *Phlox divaricata*, and *Symphytotrichum cordifolium*. Four species, *Allium tricoccum*, *Osmorhiza claytonii*, *Maianthemum stellatum*, and *Thalictrum dioicum* averaged greater than 10% colonization rates, but individual root samples within each species varied widely in the amount of AMF. The amount of AMF in nursery plant roots appeared to be influenced both by the mycorrhizal status of the herb species as well as the planting medium used in nursery production; among mycorrhizal species, colonization rates appeared lower in soilless potting mix than in outdoor production fields or pasteurized local soils.

AMF colonization rates in most herb species appeared to increase after growing in field plots for multiple years in soils affected either by garlic mustard or resident native vegetation. Only two species, *Geranium maculatum* and *Hydrophyllum virginianum* continued to show no evidence of root colonization by AMF. Among the colonized species, there were no significant differences in colonization rates between invaded and non-invaded areas at either site (Fig. 12a). In both multi-species analyses (three-year old plants at both study sites, and two- and three-year old plants at CG only), only the plant species had a significant effect on AMF colonization rates ($p < 0.0001$; Fig. 12 b, c).

In the two-site analysis, however, AMF did appear to be marginally influenced by interactions of site and invasion ($p = 0.07$; Fig. 12b), species and invasion ($p = 0.08$) (Fig. 12a), and site and species ($p = 0.06$). Overall effects of invasion on AMF colonization appeared to differ by site: at CG, AMF colonization was higher in non-invaded plots than in invaded plots, while the reverse was true at WNC. Colonization rates appeared to differ by site in non-invaded plots, while rates were similar in invaded plots at each site. While across species, there was a general, but not significant, pattern toward lower AMF

rates in non-invaded plots, colonization rates of individual herb species appeared to differ in their response to both site and invasion; for example, *Actaea rubra* and *Thalictrum dioicum* appeared to have higher AMF colonization rates in non-invaded soils at CG, but no difference between invaded and non-invaded plots at WNC. Of the species tested, only *Maianthemum racemosum* had consistently higher AMF in invaded soils than in non-invaded soils, but these effects were not significant. Across species, AMF colonization rates were slightly higher at CG than at WNC, but this pattern was only consistently observed in *A. rubra* and *T. dioicum*; within-species site differences were not significant.

Effects of Garlic Mustard Invasion on AMF Colonization in Resident Plants

As with the restored herbs, roots collected from resident (non-planted) herbs growing within a dense garlic mustard infestation and a nearby non-invaded area did not show any evidence of an invasion effect. Three species, *Eurybia macrophylla*, *Desmodium glutinosum* and *Maianthemum racemosum*, were colonized by AMF; rates of colonization differed significantly by species ($p = 0.002$), but there were no significant differences as a result of growing in invaded or non-invaded areas (Fig. 13). No AMF colonization was observed in either resident *Geranium maculatum*, nor in *Alliaria petiolata*.

Discussion

Garlic mustard invasion did not inhibit growth of restored herbs in two oak woodland sites; rather, it appeared to have a facilitative effect on herb growth, possibly due to increased soil nutrient availability in invaded areas. Garlic mustard did not appear to decrease light availability or have strong impacts on rates of AMF colonization in restored herbs. Soil nitrogen availability and herb biomass both remained high even after multiple years of garlic mustard removal, suggesting a potential “positive legacy effect” via fertilization. Post-invasion restoration of native herbs may not be inhibited by garlic mustard legacy effects, but may require management of other ecosystem stressors.

Does Garlic Mustard Impact Native Herb Species?

Garlic mustard is often implicated as a cause in native plant decline, but the hypothesis that native herbs are negatively impacted by garlic mustard was not supported by this study. Invasion did affect native plants, but the impacts varied by herb species, site, and environmental variables (light and nutrient availability). Moreover, despite these complex interactions, the overall effect of garlic mustard on native herbs was either positive or neutral, suggesting that restored native herbs benefited from garlic mustard's effects relative to the effects of non-invaded native vegetation. None of the species examined in this study had a consistent negative response to garlic mustard invasion. Although three-year-old *Allium tricoccum* appeared to respond negatively to invasion (Fig. 6c), two-year-old *A. tricoccum* was relatively unaffected (Fig. 8). Three-year-old *Geranium maculatum* appeared to have somewhat lower biomass in invaded plots compared to non-invaded plots (Fig. 6 a), but the opposite pattern was observed at WNC (Fig. 6b). *Hydrophyllum virginianum* also appeared to have opposite responses to invasion at the different study sites: both two- and three-year-old plants had lower biomass in invaded plots at WNC (Figs. 9 and 6b), but had consistently higher biomass in invaded areas at CG (Figs. 6a and 7). Thus, while the overall effect of invasion on native herbs appears to be positive or neutral, there may be site conditions and year effects that alter garlic mustard's impact on some species.

These results are consistent with other findings that herbaceous plants may be less sensitive to garlic mustard's impacts than other taxa, such as tree seedlings (McCarthy 1997, Stinson et al. 2006, Stinson et al. 2007). Garlic mustard may therefore not be responsible for native herb declines. To my knowledge, this is the first study that has reported a positive response to garlic mustard invasion. This unexpected finding suggests that garlic mustard does, in fact, affect native plant communities, but the direction, magnitude and mechanisms of impact may vary by native taxa.

Potential Mechanisms of Garlic Mustard's Impacts on Native Herbs

Multiple mechanisms have been hypothesized to explain garlic mustard's potential impacts on native plant communities, including competition and reduced light availability (Anderson et al. 1996, Dhillion and Anderson 1999, Meekins and McCarthy 1999), and soil-mediated impacts, such as direct allelopathy (Prati and Bossdorf 2004, Pisula and Meiners 2010) and indirect allelopathy resulting from reductions in beneficial mycorrhizal associations (Stinson et al. 2006, Callaway et al. 2008). The present study did not support the hypothesis that altered light availability is an important pathway of impact on the native herbs studied. Although both light availability and herb biomass were higher in plots subjected to vegetation removal treatments, there was no significant difference in light availability in invaded plots relative to non-invaded plots, in either removal or no-removal treatments. Garlic mustard did not, therefore, appear to have greater light-mediated impacts on restored herbs than resident non-invaded native vegetation. Furthermore, the fact that herb biomass tended to be higher in invaded plots than in non-invaded plots within the no-removal treatments contradicts the hypothesis that garlic mustard harms native herbs via light suppression. If the presence of garlic mustard was harming native plants by reducing light availability, we would expect to see lower biomass in invaded plots than in non-invaded plots when resident vegetation was present. However, given that many woodlands have experienced reductions in groundcover as a result of other stressors (Brewer 1980, Robinson et al. 1994, Rooney et al. 2004, Rooney and Rogers 2011), it is reasonable to expect that garlic mustard invasion does alter light availability relative to non-invaded areas when the non-invaded areas lack continuous groundcover.

Garlic mustard does not appear to be driving declines in native herbs by decreasing light availability, according to the results of this study. It should be noted, however, that all but one of the study species were summer-dominant herbs that are adapted for achieving maximum growth under a closed tree canopy. Garlic mustard bolts and achieves maximum biomass in the spring (Anderson et al. 1996), taking advantage of unutilized forest floor light before summer-dominant herbs have emerged. This

phenological niche separation appears to contribute to garlic mustard's invasive success in woodlands (Jean Engelhardt and Anderson 2011), and may also have disproportionate impacts on native spring ephemerals that share a similar light acquisition strategy (Herold et al. 2011). Only one spring ephemeral, *Allium tricoccum*, was included in this study, and it could only be analyzed at CG due to high mortality at WNC. Three-year-old *A. tricoccum* did, in fact, appear to have lower biomass in invaded plots, although there was substantial variation in biomass, and the within-species effects of invasion were not significant. Relative effects of garlic mustard versus native vegetation removal were not clearly discernible for *A. tricoccum* in this study, but the response to vegetation removal treatments appeared to be minimal. This may be due to the fact that vegetation removal was conducted in late spring and early summer and therefore likely missed the light acquisition window for *A. tricoccum*. Herold et al. (2011) found that early spring garlic mustard removal treatments benefitted spring ephemerals while summer removal treatments had minimal effects. Seasonality of garlic mustard control methods may influence the response of native plants, resulting in shifts in understory species composition.

The hypothesis that garlic mustard negatively impacts herbs through soil-mediated effects, i.e. via direct or indirect allelopathy, was also not supported by this study. The positive to neutral biomass response to invasion suggests that such antagonistic interactions are not occurring, or are outweighed by other more facilitative interactions. Direct allelopathy was not explicitly investigated in this study, but were it to impact native herbs planted as plugs or bare rootstock, we would expect to see a negative biomass response. Investigations of garlic mustard's allelopathic effects on crop species and native plants have yielded conflicting results, depending on the species tested, extracts and application levels used and other experimental conditions (McCarthy and Hanson 1998, Cipollini et al. 2008a, Cipollini et al. 2008b, Barto and Cipollini 2009a, Lankau 2010, Pisula and Meiners 2010). Allelopathic effects may be more important at the seed germination and establishment phase, which were not examined in this study (but see Chapter 3). Native species tested have exhibited moderate reductions in

germination and/or seedling growth (Prati and Bossdorf 2004, Barto et al. 2010b), though in some cases, only at extract levels that exceed those found in the field (Barto and Cipollini 2009a).

Indirect allelopathy through the suppression of beneficial AMF associations was also not evident in this study. Rates of root colonization by AMF did not differ significantly or consistently between invaded and non-invaded areas in either resident or planted herbs. Garlic mustard may not, in fact, be affecting AMF colonization rates in the herbs studied, or effects may be too subtle to be detected through the “noise” of environmental variability. Resident herbs, which were selected for this study precisely because they occurred both within and outside of a dense garlic mustard infestation, may have been biased toward disturbance-adapted AMF species that were more tolerant of garlic mustard’s effects and thus less likely to show an invasion impact (Barto et al. 2011). However, such a bias would not be expected in restored herb species planted into invaded and non-invaded areas, as neither the herbs nor their associated AMF had experienced garlic mustard’s effects prior to the experiment. Although a few species of planted herbs were already colonized at the time of planting and likely introduced new AMF into the field plots, we would still expect to see differences between invaded and non-invaded plots, if garlic mustard’s effects were inhibitory to AMF colonization. Presumably, average colonization rates would remain low or decrease in herbs planted into invaded plots, while increasing in herbs planted into non-invaded plots. However, the results of this study did not provide strong evidence that AMF colonization rates differed as a result of garlic mustard presence. Both the potency of garlic mustard’s phytochemicals and the sensitivity of AMF species to those chemicals have been found to decline over a chronosquence of garlic mustard invasion (Lankau et al. 2009, Barto et al. 2011, Lankau 2011), suggesting that the strength of the AMF-mediated pathway may lessen over time. Although the age of infestations at these study sites were unknown, Minnesota is at the westward edge of garlic mustard’s range expansion in northeastern American forests, and thus are likely relatively “young” infestations compared to most garlic mustard research;

it therefore seems unlikely that the age of infestation is responsible for the lack of AMF impact.

Although there was a slight and non-significant pattern of lower AMF in invaded plots, invasion by species and site interaction trends suggested that any potential effects on AMF may not be uniform across sites or native plant species. AMF species may differ in their response to garlic mustard's phytochemicals (Barto et al. 2011), and native plant species may differ in their response to resulting AMF species composition (Bever 2002, Klironomos 2003). Although AMF species are typically characterized as generalists, species-specificity in the plant-AMF relationship may be more common than previously thought (McGonigle and Fitter 1990, Dhillion 1992, Bever et al. 1996, Vandenkoornhuyse et al. 2003, Smith and Read 2008). Studies of garlic mustard's potential effects on AMF composition have yielded mixed results. Garlic mustard extracts applied in a greenhouse assay were found to affect AMF growth but not AMF richness or composition (Koch et al. 2011). In a field study, however, Burke (2008) found no significant differences in AMF root colonization rates in three woodland herb species collected from invaded and non-invaded areas, but did find significantly different AMF species composition within the roots of *Maianthemum racemosum*, suggesting that garlic mustard may selectively suppress AMF. Although analysis of AMF species composition was beyond the scope of this study, it may explain the lack of an invasion response of colonization rates, as well as the apparent interactions with site and herb species. AMF species that are tolerant of garlic mustard may increase or maintain abundance following invasion, while more sensitive AMF species may decline (Barto et al. 2011, Lankau 2011). Depending on the existing AMF species composition at a given site, and the species specificity for individual AMF and plant species, invasion may result in either higher, lower or unaffected AMF colonization rates. Furthermore, in disturbed woodlands, AMF communities may already be disrupted via other environmental stressors. Exotic earthworms, which were present in both of these study sites, have also been implicated in AMF disturbances (Bohlen et al. 2004). Effects of garlic mustard on

AMF colonization rates may not be apparent in woodlands that are similarly impacted by other invasions.

Regardless of garlic mustard's potential impacts on AMF colonization rates or composition, there was no indication in this study that such effects negatively impact planted herbs. Herb biomass patterns did not negatively correlate with AMF trends either at the site or species level. For example, although CG had slightly higher overall AMF colonization rates than WNC and higher AMF colonization in non-invaded plots compared to invaded plots, herb biomass was, on average, higher at WNC than at CG, and higher in invaded plots than in non-invaded plots at CG. Similarly, herb species that appeared to have somewhat reduced AMF in invaded plots, such as *Actaea rubra* and *Thalictrum dioicum* at CG, appeared to have higher—not lower—biomass in invaded plots, which suggests either that AMF are not benefiting native herbs, or that other positive effects of invasion are outweighing any negative effects via decreased AMF.

The unexpected positive response of planted native herbs to garlic mustard invasion is most reasonably explained by the elevated nutrient availability in invaded areas. In this study, I observed higher availability of NO_3^- , NH_3^+ , and P in invaded plots, which is consistent with findings of Rodgers et al. (2008b), who also reported consistently and significantly higher levels of these nutrients (as well as calcium, magnesium and soil pH) in invaded plots compared to non-invaded plots. While I cannot conclusively demonstrate that garlic mustard was responsible for the observed differences in soil nutrients, Rodgers et al. (2008b) reported that, while nutrients were consistently higher in invaded plots within sites, not all invaded areas had higher nutrient availability than all non-invaded areas, which would be expected if garlic mustard were restricted to growing in the most fertile sites. My observations support this circumstantial evidence: garlic mustard spread rapidly in both field sites over the course of my study, and by the third year garlic mustard had successfully invaded the “non-invaded areas”, which indicates that garlic mustard invasion was not limited by lower nutrient availability in the non-invaded sites. Invasive plant species have been found to increase nutrient availability and rates of nutrient cycling relative to co-occurring native plants through a variety of

mechanisms (Ehrenfeld 2003). Although the mechanisms by which garlic mustard increases nutrient availability are as of yet unclear, increased rates of litter decomposition stimulated by inputs of high-nutrient content rosette leaves appears to contribute to garlic mustard's positive effects on N availability (Rodgers et al. 2008b); similar increases in decomposition rates and N availability have been reported in invasive woodland trees and shrubs (Ehrenfeld et al. 2001, Heneghan et al. 2002).

The impacts of garlic mustard's fertilization effect on native herbs and woodland communities have not previously been explored, but this study suggests that it may benefit planted herbs. Similar facilitative effects of nutrient-enriching invasive plants on native vegetation have been reported in grassland systems (Van Riper and Larson 2009). Although nutrient data was not available for all of the analyses in this study, inclusion of the nutrient covariate reduced the apparent impact of invasion on herb biomass. This effect was most clearly observed in the analysis of two-year-old plants at CG: when the analysis was run without the nutrient covariate, multiple herb species were found to have significant positive responses to garlic mustard invasion. However, when the nutrient covariate was included, essentially separating out the potential fertilization effect of garlic mustard from other pathways of impact, the differences between invaded and non-invaded areas were no longer significant for any herb species. While garlic mustard's ability to enhance nutrient availability was not explicitly tested in this study, these results suggest that the fertilization effect may be an important pathway of impact for the woodland understory community. In particular, the increase in nitrogen availability may result in higher biomass production of in forest herbs (Abrams and Dickmann 1983, Turkington et al. 1998, Anderson 2003).

Native herbs may respond more strongly to garlic mustard's effects on nitrogen availability than its effects on phosphorus availability; productivity in North American forests is generally not phosphorus-limited, but historically nitrogen limitation is more common (Vitousek and Howarth 1991). However, in the past century, fertilization effects at the regional scale due to industrial nitrogen deposition and agricultural run-off may also be altering woodland nutrient dynamics (Vitousek et al. 1997, Matson et al. 2002,

Gilliam 2006). Nitrogen availability in forests tends to be patchy, and effects on herb growth often interact with light and moisture availability, as well as herbivory (Anderson 2003). Phosphorus and nitrogen were both elevated in invaded areas at CG and WNC, but phosphorus levels did not differ significantly by study site, while site differences in nitrogen suggested a potential homogenizing effect of garlic mustard: nitrogen levels in non-invaded areas at CG were significantly lower than non-invaded areas at WNC, while invaded areas at both sites had comparably high levels of nitrogen and did not differ significantly. The NMS analysis that combined multiple measurements of NO_3^- , NH_3^+ , P as well as pH—all of which have been shown to be influenced by garlic mustard (Rodgers et al. 2008b)—produced a synthetic axis largely defined by nitrate, reflecting the differences in nitrate availability across both study sites and plant composition (invaded vs. non-invaded). When this synthetic nutrient axis was included in analyses of biomass responses to garlic mustard invasion, it appeared to have a greater influence on results of CG plants than WNC plants, which is reasonable, given the greater magnitude of difference in NO_3^- availability between invaded and non-invaded areas at CG.

The results of this study suggest that garlic mustard may have minimal impacts on native herbs via the reduction of their mycorrhizal associates. For restored herbs, garlic mustard's fertilization effects may outweigh any costs of disrupted mycorrhizal associations. One of the important benefits that AMF provide their plant hosts is increased access to limited soil nutrients, phosphorus and nitrogen in particular (Barea et al. 1987, George et al. 1992, Siqueira and Saggin-Júnior 2001, Smith et al. 2003b, Jia et al. 2004). However, in conditions of high nutrient availability, some plants may not require AMF to acquire nutrients, and the AMF relationship may become irrelevant, or even parasitic if the carbon costs of maintaining the relationship outweigh the benefits received (Johnson et al. 1997, Rowe et al. 2007). Thus, by increasing nutrient availability, garlic mustard may decrease the importance of AMF to native herbs, or even provide an additional benefit by making nutrients more freely available without an associated “carbon tax”. Future research that aims to manipulate garlic mustard's

multiple pathways of impact may yield a clearer understanding of their relative importance to different woodland taxa under varying environmental conditions.

Do Impacts Persist Following Garlic Mustard Removal?

To anticipate likely outcomes of garlic mustard control, it is important to consider the extent to which garlic mustard's impacts persist following complete or partial removal. In this study, I hypothesized that removal of garlic mustard would benefit planted herbs less than removal of non-invaded native vegetation due to a persistent soil legacy effect that continued to inhibit herb growth following removal. If the legacy effect persisted for multiple years, I expected that herbs planted into plots with multiple years of garlic mustard removal would continue to have low biomass. I found that although garlic mustard does appear to have a soil legacy effect, it may be a facilitative legacy that resulted in higher biomass of planted herbs. There was an invasion by removal interaction, as expected, but rather than showing less benefit than native vegetation removal, garlic mustard removal appeared to provide an increased benefit. This increased benefit did not appear to result from greater release from competition in invaded plots relative to non-invaded plots, because herb biomass was also higher in invaded plots when vegetation was left intact. Instead, it appeared that herbs planted into garlic mustard removal plots benefitted both from competitive release or increased light availability, and from the increased availability of nutrients, likely resulting from garlic mustard invasion.

Nitrate availability increased significantly in response to vegetation removal treatments, and the magnitude of increase appeared to be higher in invaded plots compared to non-invaded plots (Fig. 5b). Nitrate levels were still elevated in study plots after three years of sustained garlic mustard removal treatments (Fig. 5d), despite the fact that the plots were densely planted with native herbs (30 plants/m²). Native herbs varied in their response to vegetation removal treatments, possibly reflecting differences in shade tolerance and light acquisition strategies (Sparling 1967, Givnish 1982, Mitchell and Woodward 1988, Neufeld et al. 2003), but in several cases herbs demonstrated a

greater positive response to garlic mustard removal than to native vegetation removal. In six out of eight species tested, biomass was higher in garlic mustard removal treatments compared to no-removal treatments, even when planted into plots from which garlic mustard had been removed one or two years prior to planting (Fig. 11). However, there was indication that the benefits of removal might begin to dissipate three years after removal; although biomass differences between two- and three-year removal plots were not significant, at CG, four species had slightly lower biomass in three-year removal plots than in two-year removal plots. Whether this pattern reflects the subsidence of garlic mustard's legacy effect or other sources of variability cannot be discerned, as soil nutrients were not measured during the year of plant harvest, and the study was not continued beyond three years.

Restored native herbs appeared to benefit from garlic mustard's soil-mediated effects both when growing in the presence of the living invader, and even more so in soils formerly occupied by the invader. Native herb responses to partial garlic mustard removal might therefore depend on whether the remaining low densities of garlic mustard exert a net positive effect due to elevated nutrient availability, or a net negative effect due to competition. Since nitrate availability appeared to remain elevated for at least three years following garlic mustard removal, the nutrient and biomass responses to partial removal treatments might be difficult to distinguish from the legacy of removed garlic mustard in a short-term study such as this. Unfortunately, nutrient data was not collected in partial removal plots, so the mechanisms of impact in incomplete removal plots cannot be adequately addressed in this study, and herb biomass responses were somewhat inconclusive. I originally hypothesized that if garlic mustard's negative impacts persisted at low densities, partial removal plots would be more similar to no-removal plots, in terms of herb biomass, than to full-removal plots. Given that herbs in this study responded positively to garlic mustard, we might instead expect partial removal plots to be more comparable to full-removal plots, as herbs would benefit both from the increased nutrient availability and decreased competition. Although removal effects were only significant at WNC, herb biomass in partial removal plots was generally either

intermediate between full and no-removal treatments, or higher than biomass in full removal plots (Fig. 10).

Whether partial or full removal treatments yielded higher biomass appeared to differ by herb species, site and plant age/planting-year effects, which suggests that outcomes of different garlic mustard control targets and methods might not be uniform across sites or years. Species that experience greater benefit in partial removal treatments (e.g. *Actaea rubra*) might have stronger responses to incomplete garlic mustard removal, including biocontrol. Stinson et al. (2007) found that some tree species exhibited a more positive response to 50% reductions in garlic mustard than to full removal and concluded that since outcomes of incomplete removal were comparable to full removal, complete eradication of garlic mustard may neither be necessary or cost-effective. Some species may experience a “double-benefit” of incomplete removal resulting from partial release from competition plus increased nutrient availability.

Implications for Post-Invasion Restoration

In order to anticipate likely outcomes of garlic mustard control, it is important to understand the extent to which garlic mustard is driving changes in woodland plant communities, whether garlic mustard’s impacts persist at low densities, and whether post-invasion recovery of the plant community is limited by garlic mustard’s legacy effects or a biotic legacy of dispersal limitation. Planted herbs in this study were not negatively impacted by garlic mustard invasion. Herb biomass responses to garlic mustard invasion were either neutral or positive, and neither forest-floor light availability nor AMF colonization rates were significantly reduced by invasion. Garlic mustard’s fertilization effect may have a greater influence on woodland herb growth than either light- or AMF-mediated impacts. Although other taxa (e.g. tree seedlings) have been found to be impacted by garlic mustard invasion (Stinson et al. 2006), this study suggests that garlic mustard invasion is not a primary driver of decline of woodland herb communities.

Garlic mustard invasion may, however, have broader regional impacts. Site by invasion interaction trends in this study suggested possible homogenization of biotic and

abiotic features of woodland soil communities. AMF colonization and nutrient availability differed between non-invaded areas within each site, but invaded areas at each site were similar. Garlic mustard's fertilization impacts may disproportionately affect nutrient-poor sites and potentially shift the competitive balance among plant species. Many exotic and ruderal species thrive in nutrient-enriched conditions (Milchunas and Lauenroth 1995); these species may ultimately displace species that are tolerant of low-nutrient environments (Tilman 1987). Such facilitative effects on exotic species has been observed in studies of other nutrient-enriching plant invaders (Carino and Daehler 2002, Yelenik et al. 2004). While the long-term implications of garlic mustard's nutrient enrichment are not yet clear, regional diversity may decline despite local increases in productivity (DiTommaso and Aarssen 1989, Jefferies and Maron 1997, Matson et al. 2002, Clark and Tilman 2008). Such impacts may be exacerbated by similar homogenizing effects of other invasions, deer, and anthropogenic stressors (Gordon 1998, Horsley et al. 2003, Bohlen et al. 2004, Rooney et al. 2004, Frelich et al. 2006, Greiner et al. 2012).

Garlic mustard's fertilization effect may leave a "positive" legacy, resulting in increased herb biomass following garlic mustard removal. Herb biomass and nitrate availability were both generally higher in garlic mustard removal plots even after multiple years of removal. Thus it appears nutrient enrichment may continue to enhance native plant productivity following garlic mustard removal. An important caveat, however, is that nitrate is highly mobile in soils and may have remained high in study plots due to leaching from surrounding invaded areas. If elevated nitrate levels were due only to leaching, we might expect to see site differences in the response to garlic mustard removal, as the steep, sandy ravine of CG would likely be more susceptible to leaching than soils at WNC, and there was little evidence of this. Further research is needed to better understand the spatial and temporal dynamics of nutrient responses to garlic mustard removal. Nevertheless, there is no indication in this study that restoration of native herbs will be inhibited by post-invasion soil legacies. Native plants in other systems invaded by nitrogen-enrichers have been found to recover quickly following

removal of the invader, taking advantage of the increased light and nitrogen availability (Hughes et al. 2012). Immediate planting may be advisable in order to take advantage of the flush of nutrients, particularly because unutilized resources may contribute to site invasibility (Davis et al. 2000, Gilliam 2006). Nitrate levels remained high in study plots even after a fairly dense restoration planting; thus continued monitoring for reinvasion of exotics is recommended following post-invasion restoration.

Once garlic mustard is established in a woodland site, complete eradication may not be feasible, as successful control requires many years of sustained labor-intensive removal efforts. Because of the lack of effective control methods for large infestations, many land managers and property owners are hopeful that biocontrol will be the solution for managing extensive garlic mustard infestations. Native plant community recovery, however, may depend on whether garlic mustard's impacts persist at the reduced densities achieved by biocontrol or conventional control methods. Although the effects of incomplete removal were somewhat inconclusive in this study, it did appear that native herb species may differ in whether they benefit more from full or partial removal. While degree of removal treatment effects were not significant, herbs did generally appear to benefit from either full or partial garlic mustard removal; thus this study does not support the hypothesis that native herbs will be inhibited by garlic mustard at low densities. Biocontrol and other incomplete control methods may thus be sufficient for community recovery.

Overall patterns of herb responses to garlic mustard invasion and control were somewhat difficult to discern in this study because of considerable site and species interactions in nearly every analysis. Although two study sites is not sufficient for distinguishing broad site-level trends, this study does suggest that the impacts of garlic mustard and its removal may not be uniform across invaded sites and environmental conditions, and so caution is advised in assuming the patterns found at these study sites apply to the full extent of garlic mustard's invaded range. Species-specific responses to garlic mustard invasion and removal are not unexpected; native species are often found to differ in their sensitivity to invasion impacts, legacy effects, and even invasion control

methods (Holmes et al. 2000, Perry et al. 2005, Butler et al. 2006, Bush et al. 2007, Jordan et al. 2008, Hahn et al. 2011, Herold et al. 2011). This may be particularly true of invasive species that affect communities via multiple mechanisms. Garlic mustard is certainly not unique in this regard (Gordon 1998, Levine et al. 2003); for example, invasive shrubs in prairies and woodlands have been found to suppress herbaceous vegetation both by reducing light availability and altering soil fertility and biota (Woods 1993, Heneghan et al. 2006, Klionsky et al. 2010, Pierce and Reich 2010, Greene and Blossey 2011). Community level responses to invasion and control thus depend on the sensitivity of individual species to each mechanism of impact. Tree seedlings, for example, appear to be more sensitive to garlic mustard's AMF-mediated impacts than herbaceous species, and spring ephemerals may be more sensitive to light-mediated impacts and seasonality of garlic mustard removal than summer dominants. Both initial garlic mustard invasion and subsequent removal may therefore result in "winners" and "losers" among native plant species (McKinney and Lockwood 1999, Wiegmann and Waller 2006).

Woodland herbs in general appeared to be among the "winners", as planted herbs responded positively both to the presence and removal of garlic mustard. This study did not, however, assess natural recovery or restoration by seed. Herb regeneration in disturbed woodland may be limited by availability of propagule sources (Brudvig et al. 2011). Some herb species may be more sensitive to garlic mustard's impacts during seed germination and establishment phases; for example, seed germination may be particularly sensitive to allelopathy, and potentially to AMF-mediated effects, as herbs planted by seed are not colonized by AMF prior to planting. Herbs cultivated in local soils unaffected by garlic mustard may serve as an AMF inoculum source for restored woodlands, although our knowledge of which AMF species and conditions result in benefits to native plants is currently too limited to count on this method for strategic restoration of AMF communities (Hart and Trevors 2005). Regardless, while restoration by plugs and bare rootstock is a more expensive and labor-intensive approach than seeding, it is likely to be a more effective way to rapidly establish a ground cover of

native woodland herbs, which tend to have complex germination requirements and slow growth. Such rapid restoration may be particularly important given the high resources availability and “vacant niche” left behind following garlic mustard control.

Planting herbs into formerly-invaded sites may be an important step in limiting reinvasion and restoring native plant communities (Vidra et al. 2007), and fortunately, this process may not be inhibited by garlic mustard’s soil legacy. However, the fact that garlic mustard does not appear to be responsible for declines in native herbs suggests that its control may not be sufficient for their recovery (MacDougall and Turkington 2005, Bauer 2012). Herbivory by deer and exotic slugs, and the ecosystem engineering effects of earthworms, have all been implicated as potential causes of native herb decline (Hahn et al. 2011). Field plots in this study were fenced to minimize deer herbivory, and observations of herbivory on resident herbs and planted herbs following removal of fences strongly suggested that native plant restoration and recovery might be inhibited by deer (pers. obs.). This is particularly important given that in some studies, herb biomass only increased in response to fertilization when protected from herbivory (Anderson 2003). Slugs and earthworms were common in my study sites and frequently observed within study plots, and slug herbivory was observed on planted herbs. Slugs and earthworms appeared to be especially abundant at WNC, possibly due to the lower sand content of the soils. Their abundance may provide an explanation for the high mortality rates of several herb species at this site. *Symphytotrichum cordifolium*, for example, which had extremely poor survival both in this and another study conducted at WNC (Knight 2006), has been found to be strongly impacted by slug herbivory (Hahn et al. 2011). Hahn and Dornbush (2012) also found *S. cordifolium* was only sensitive to competitive pressure from garlic mustard when also affected by slug herbivory, while impacts of slugs were observed even in the absence of garlic mustard. (Unfortunately, mortality of *S. cordifolium* at WNC was so high, the potential impacts of invasion could not be assessed in the present study.) On the other hand, *Desmodium glutinosum*—one of the most abundant herbs at WNC (L. Van Riper, unpublished data), both in invaded and non-invaded areas—appears to experience minimal slug herbivory (Hahn et al. 2011).

Other environmental stressors may therefore exert greater influence on the composition of woodland herb communities than garlic mustard invasion, which appears to be more of a “back-seat driver” of ecological change (Bauer 2012). Identifying the primary drivers of change and elucidating the potential interactions of multiple stressors in woodland plant communities will be critical for developing comprehensive approaches to restoration.

Table 2-1. Native species included in the assessment of AMF in resident plants and in the restoration study. The number of plants per species per plot is indicated for the restoration study, as well as the form planted (bare rootstock or plugs) and month harvested (in 2009 unless otherwise indicated). Roots analyzed for AMF colonization were either from three-year-old plants at both study sites (Y1 = planted in 2006); two- and three-year-old plants at CG only (Y1 & 2 = planted in 2006 and 2007); or two-year-old plants at CG-only (Y2). Although two forms of *M. racemosum* were planted in Year 1, only data from the plug-planted were included in the multispecies analysis (for consistency with Year 2 analysis). A separate analysis comparing results of *M. racemosum* planted by bare rootstock and plugs (proc Mixed; SAS 9.2): bare rootstock plants had significantly greater biomass than plugs ($p = 0.02$), but responses to invasion and removal treatments were unaffected by plant form.

Species	Common name	Family	Studies		Restoration Study			
			AMF	Rest.	#/plot	Form	Harvest month	Roots Analyzed
<i>Actaea rubra</i> (Aiton) Willd. ^a	red baneberry	Ranunculaceae		x	2	bare root	Aug.	Y1
<i>Allium tricoccum</i> Aiton	wild leek	Liliaceae		x	3	plug	May	Y1 &2
<i>Desmodium glutinosum</i>	pointed-leaf tick trefoil	Fabaceae	x					
<i>Eurybia macrophylla</i> (L.) Cass.	bigleaf aster	Asteraceae	x					
<i>Geranium maculatum</i>	wild geranium	Geranaceae	x	x	3	bare root	Aug.	Y1
<i>Hydrophyllum virginianum</i> L.	virginia waterleaf	Hydrophyllaceae		x	3	plug	June	Y1
<i>Maianthemum racemosum</i> (L.) Link ssp. Racemosum	solomon's plume	Liliaceae	x	x	4 ^b	plug & bare root	July	Y1
<i>Maianthemum stellatum</i> (L.) Link	starry solomon's plume	Liliaceae		x	2	bare root	July	None
<i>Mitella diphylla</i> L.	bishop's cap	Saxifragaceae		x	3	plug	Aug. ^d	Y1
<i>Osmorhiza claytonii</i>	sweet cicely	Apiaceae		x	3	plug	July	None
<i>Phlox divaricata</i> L.	wild blue phlox	Polemoniaceae		x	3	plug	July	Y1 &2
<i>Solidago flexicaulis</i> L. ^c	zig-zag goldenrod	Asteraceae		x	3	plug	Sept.	Y2
<i>Symphotrichum cordifolium</i> (L.) G.L. Nesom	heart-leaved aster	Asteraceae		x	3	plug	Sept.	Y1 &2
<i>Thalictrum dioicum</i> L.	early meadow-rue	Ranunculaceae		x	2	plug	Aug. ^d	Y1

^a planted in 2006 only (harvested at 3 years) ^b two plugs and two bare root plants per plot

^c planted in 2007 only (harvested at 2 years) ^d biomass and root sample harvested in 2008.

Table 2-2. Summary of environmental variables by site and invasion. With the exception of soil texture, all data reported are natural log-transformed. Least squares means and standard error (s.e.) were calculated with Anova (proc glm; SAS 9.2). Significant treatment factors and interactions ($p < 0.05$) are indicated.

Site: Invasion:	CG				WNC				p < 0.05	
	Inv		Non		Inv		Non			
Variables	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.		
Light (DIFN)	-3.89	0.09	-3.61	0.12	-2.94	0.08	-2.94	0.12	Site	
Soil texture	% sand	90.42	1.46	89.90	1.60	71.77	1.46	74.91	1.60	Site
	% clay	1.67	0.45	2.00	0.49	4.83	0.45	3.50	0.49	Site
	% silt	7.91	1.20	8.10	1.32	23.40	1.20	21.60	1.32	Site
Soil moisture	1.69	0.06	1.58	0.06	2.87	0.06	2.67	0.06	Site, Invs	
pH (1:2)	1.75	0.01	1.74	0.01	1.64	0.01	1.61	0.01	Site	
P ($\mu\text{g P/g resin/day}$)										
May-Jul. 2007	-0.96	0.15	-2.15	0.15	-1.47	0.15	-1.81	0.14	Site*Invs	
Aug.-Sept. 2007	-0.79	0.18	-1.35	0.17	-0.71	0.17	-1.14	0.18	Invs	
May-Aug. 2008	-1.34	0.13	-2.16	0.12	-1.27	0.13	-1.65	0.12	Site*Invs*Rem	
NO3 ($\mu\text{g NO}_3\text{/g resin/day}$)										
May-Jul. 2007	0.83	0.22	-0.13	0.22	0.79	0.22	1.22	0.21	Site*Invs*Rem	
Aug.-Sept. 2007	1.55	0.23	-0.09	0.22	1.90	0.22	1.30	0.23	Site*Invs	
May-Aug. 2008	1.05	0.22	-0.16	0.22	1.09	0.22	0.54	0.22	Invs, Rem	
NH3 ($\mu\text{g NH}_3\text{/g resin/day}$)										
May-Jul. 2007	-1.35	0.26	-2.70	0.26	-1.95	0.26	-1.74	0.25	Site*Invs	
Aug.-Sept. 2007	-1.88	0.17	-2.08	0.16	-1.33	0.16	-1.25	0.17	Site*Rem	
May-Aug. 2008	-1.68	0.26	-2.77	0.26	-2.12	0.26	-2.33	0.26	Invs	

Table 2-3. Percent AMF root colonization in nursery plant roots prior to planting in Restoration Study. Herb species planted in 2007 Restoration Study. Planting medium used by native plant nurseries: SPM = soilless potting mix; PLS = pasteurized local soil; OPB = outdoor production beds. Root samples from five individual plants per species were examined.

Species	Planting Medium	# roots colonized	% AMF (mean)	S.E.	Range (%)
<i>Allium tricoccum</i>	SPM + PLS	4	14.38	9.18	0 - 55
<i>Geranium maculatum</i>	OPB	0	0.00	0.00	0
<i>Hydrophyllum virginianum</i>	SPM + PLS	0	0.00	0.00	0
<i>Mainthemum racemosum</i>	SPM + PLS	1	0.20	0.18	0 - 1
<i>Mainthemum stellatum</i>	OPB	5	66.56	4.46	54 - 83
<i>Mitella diphylla</i>	SPM + PLS	0	0.00	0.00	0
<i>Osmorhiza claytonii</i>	SPM + PLS	4	16.30	5.49	0 - 33
<i>Phlox divaricata</i>	SPM + PLS	2	3.24	2.17	0 - 12.5
<i>Solidago flexicaulis</i>	SPM	0	0.00	0.00	0
<i>Symphotrichum cordifolium</i>	SPM	1	0.19	0.17	0 - 0.95
<i>Thalictrum dioicum</i>	SPM + PLS	5	25.99	11.91	2 - 68

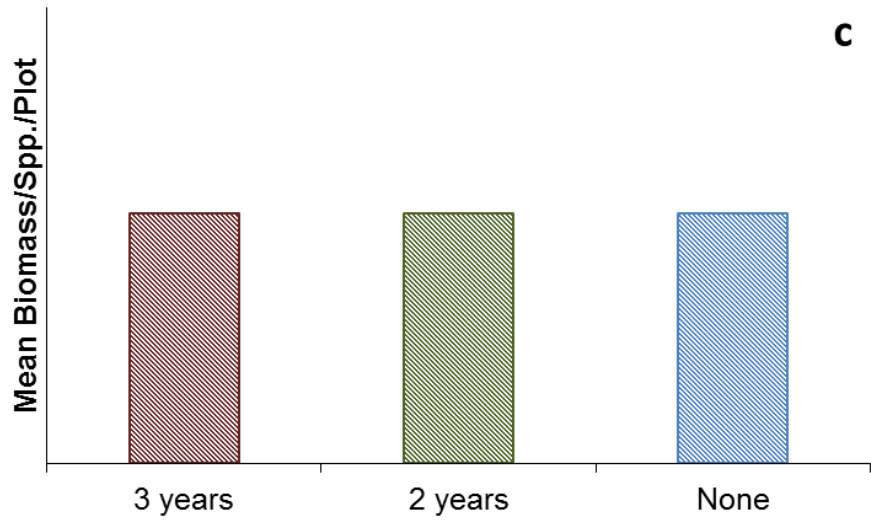
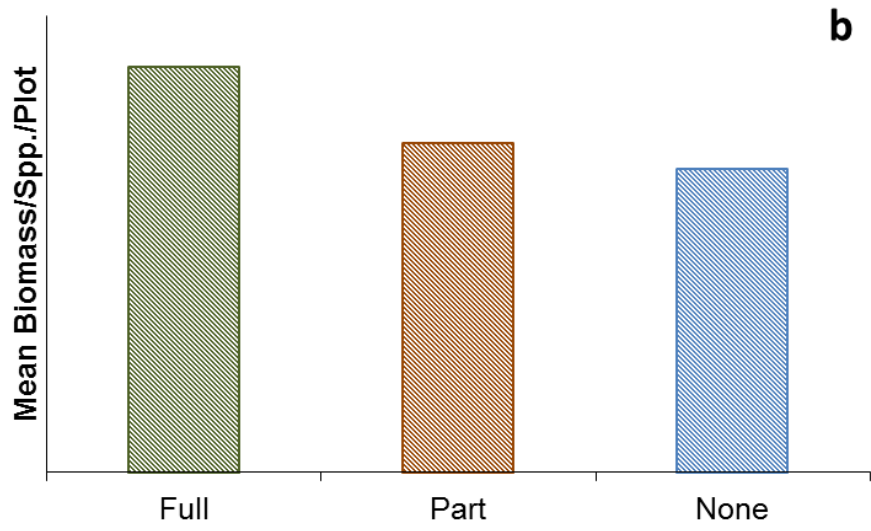
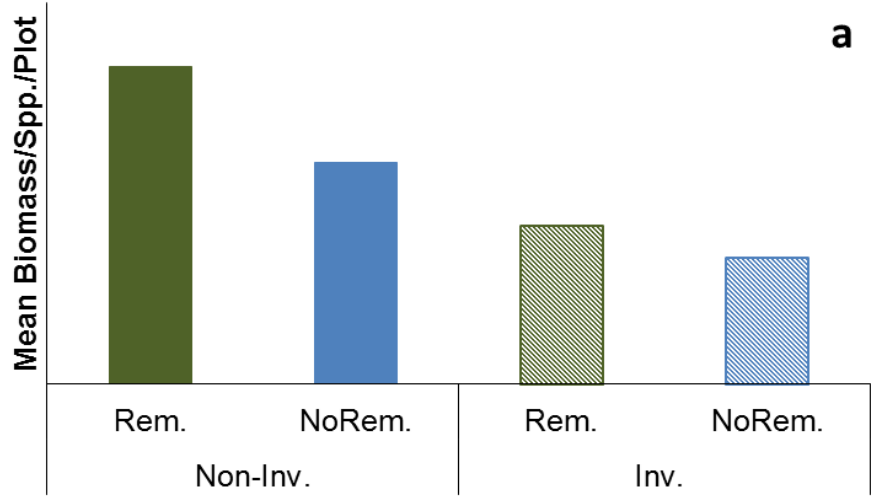


Figure 2-1. Predicted herb biomass response to garlic mustard presence and removal. **a)** Invasion & Removal: Herbs planted into garlic mustard-invaded plots were expected to have lower growth (biomass) than those planted into non-invaded soils, and they were expected to benefit less from vegetation removal than herbs growing into plots from which native vegetation was removed (an invasion by removal interaction), indicating a soil legacy effect. **b)** Degree of Removal: Herbs planted into partial-removal (low density) plots were expected to have lower biomass than full removal plots (comparable to no-removal plots) if the effects of garlic mustard persist at low densities. **c)** Duration of Removal (legacy): Biomass of herbs planted into plots with either two or three years of garlic mustard removal at the time of harvest were not expected to have higher biomass than plots with no removal if garlic mustard's soil legacy effect continued to inhibit growth.

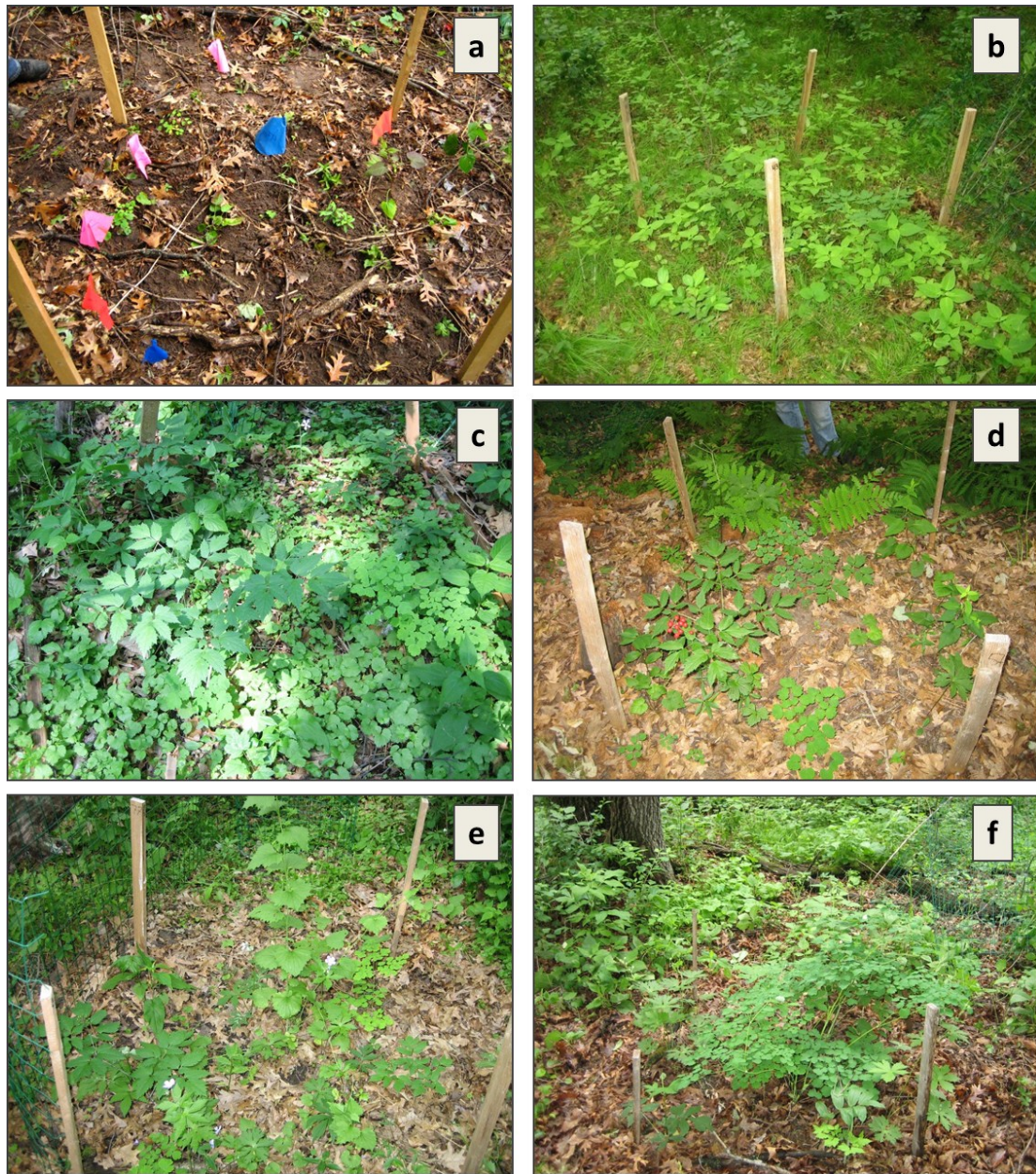


Figure 2-2. Planted field plots and removal treatments. **a)** Fall planting in invaded/partial removal plot at WNC, 2006; flags indicate location of herbs planted as bare rootstock; **b)** Non-invaded/no-removal plot at CG, 2007 (one year after planting); **c)** Invaded/no-removal plot at CG, 2007 (one year after planting); **d)** Non-invaded/full-removal plot at WNC, 2007 (one year after planting); **e)** Invaded/partial-removal plot at WNC, 2007 (one year after planting); **f)** Non-invaded/full-removal plot at WNC, 2009 (three years after planting).

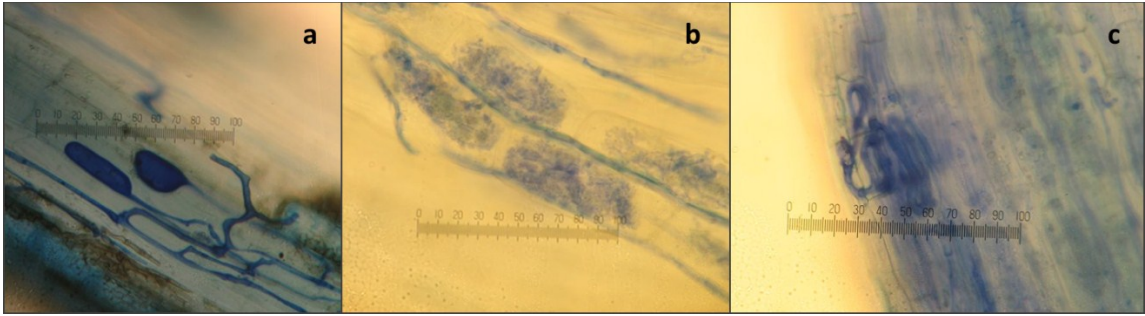


Figure 2-3. Examples of AMF structures used to determine AMF presence in native plant roots, stained with aniline blue and viewed at 200x magnification with a bright field microscope; **a)** vesicles and hyphae; **b)** arbuscules; **c)** coiling hyphae.

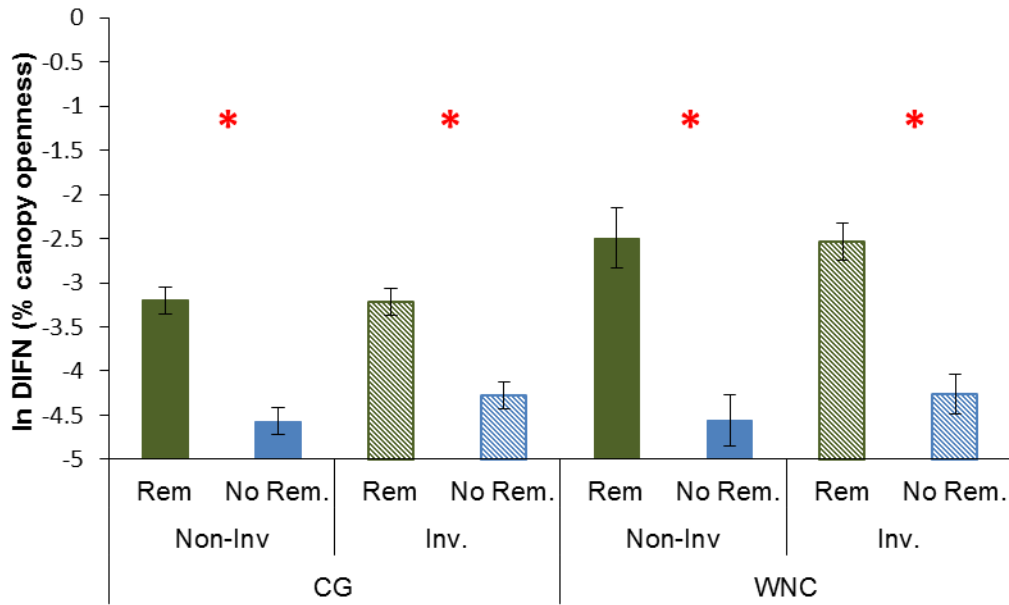


Figure 2-4. Light availability (DIFN) at the forest floor by invasion (invaded and non-invaded) and vegetation removal treatments (full removal and no removal) at two study sites: CG and WNC. Forest floor light availability did not differ significantly between invaded and non-invaded plots at either site but was affected by removal treatments. At CG the removal effect interacted with above-plot light levels ($p < 0.0001$); light availability at the forest floor increased along with increasing above-plot light in removal plots but not in no-removal plots; at WNC the removal main effect was nearly significant ($p = 0.07$) without treatment interactions. Bars represent least squares means \pm 1 SE. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments at each site.

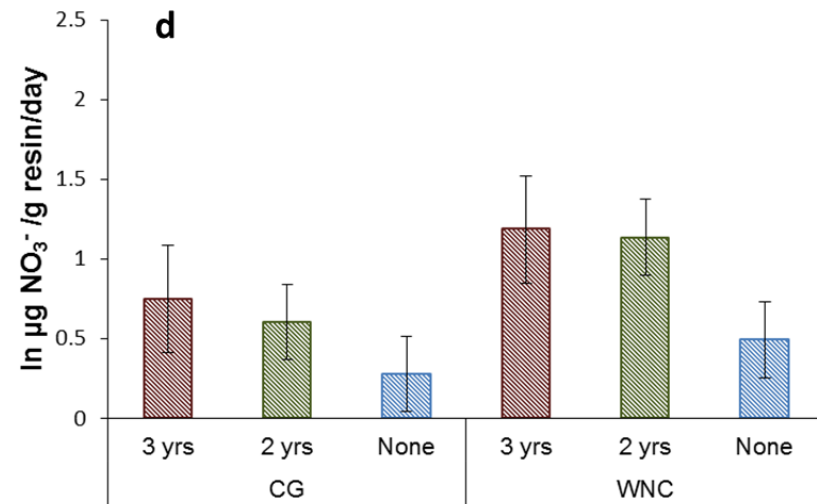
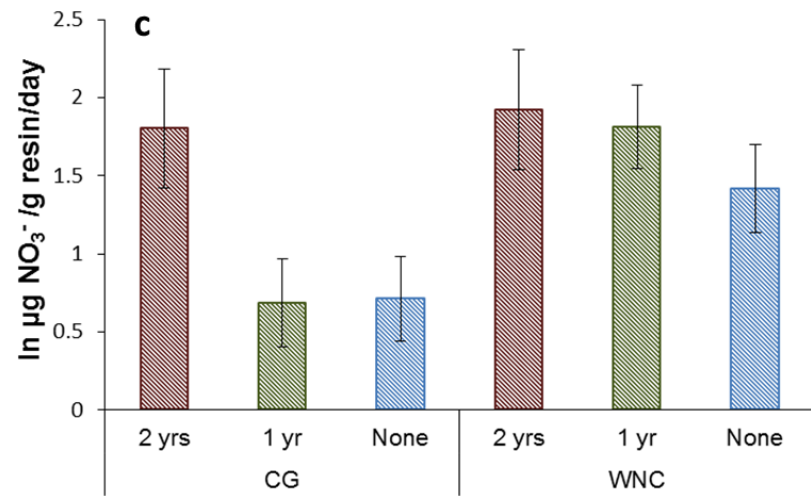
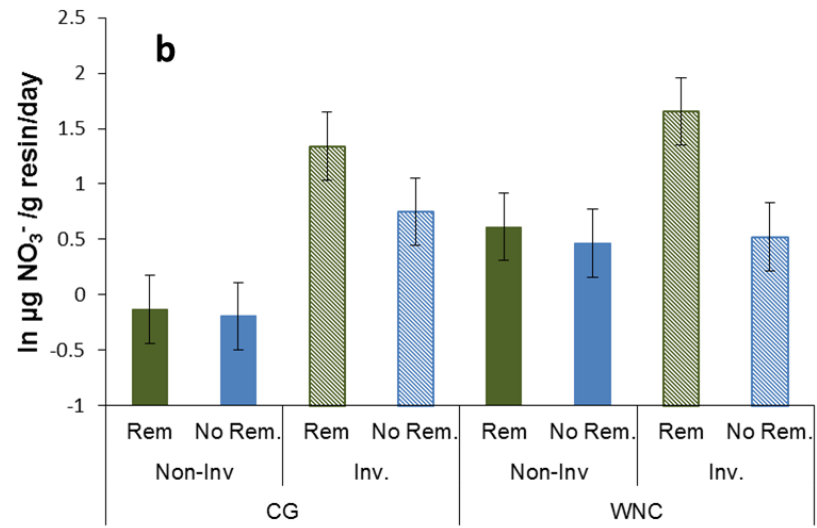
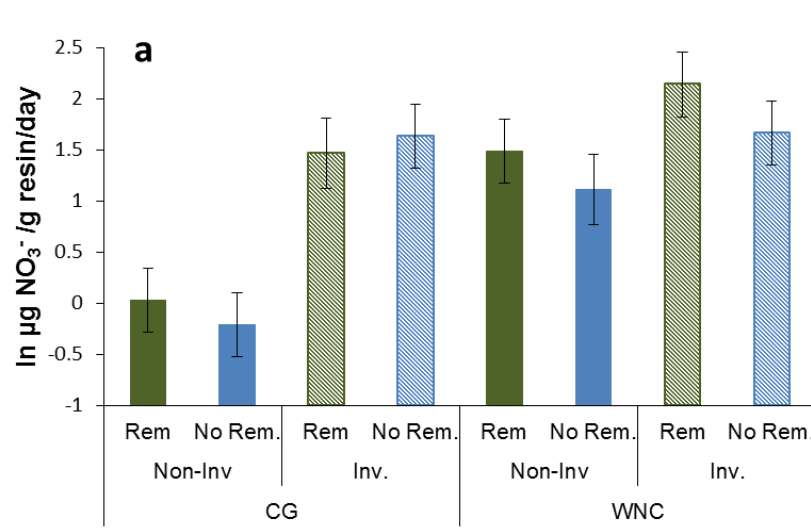


Figure 2-5. Nitrate availability by site, invasion and removal treatment. **a)** Differences by invasion (invaded and non-invaded) and removal (full and none) in late summer 2007 (after one season of vegetation removal). Invasion by site interaction was significant ($p = 0.03$); NO_3^- was significantly higher in invaded areas at CG ($p < 0.0001$) and nearly significant at WNC ($p = 0.07$); **b)** Differences by invasion and removal in summer 2008 (after two years of removal); Invasion and removal effects were significant ($p = 0.0002$ and 0.03 , respectively); **c)** Differences in nitrate availability in response to duration of garlic mustard removal in late summer 2007; NO_3^- differed significantly by site ($p = 0.01$) and nearly significantly by removal treatment ($p = 0.06$); **d)** Differences by duration of removal in summer 2008; site and main removal effects were not significant ($p = 0.08$ and 0.07 , respectively). Bars represent least squares means ± 1 SE.

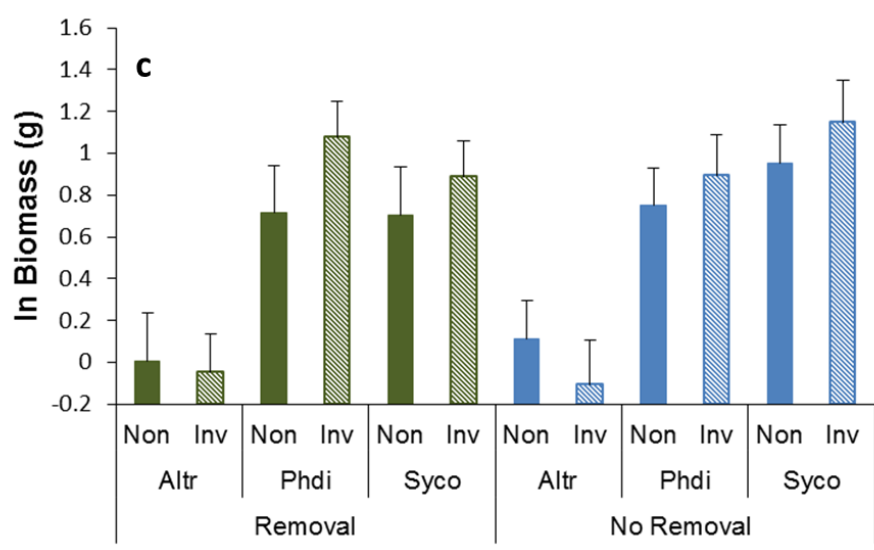
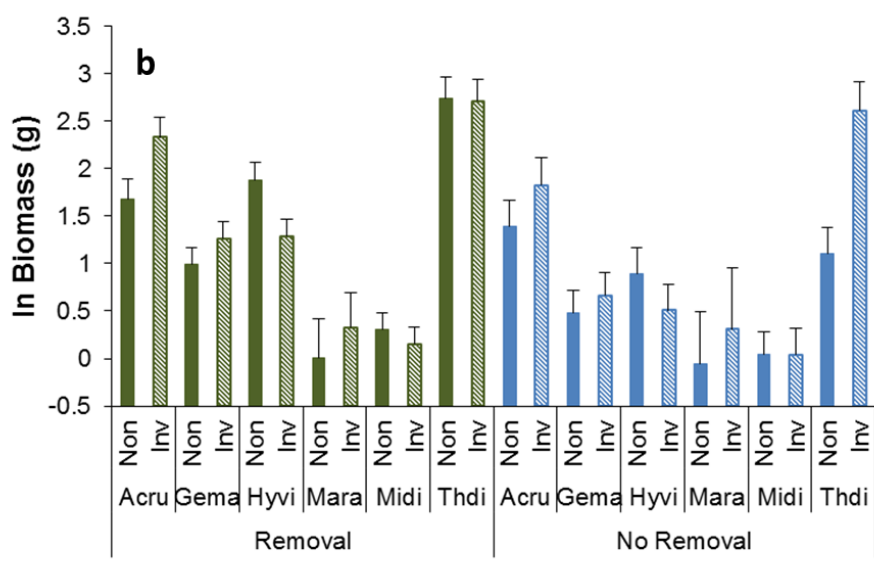
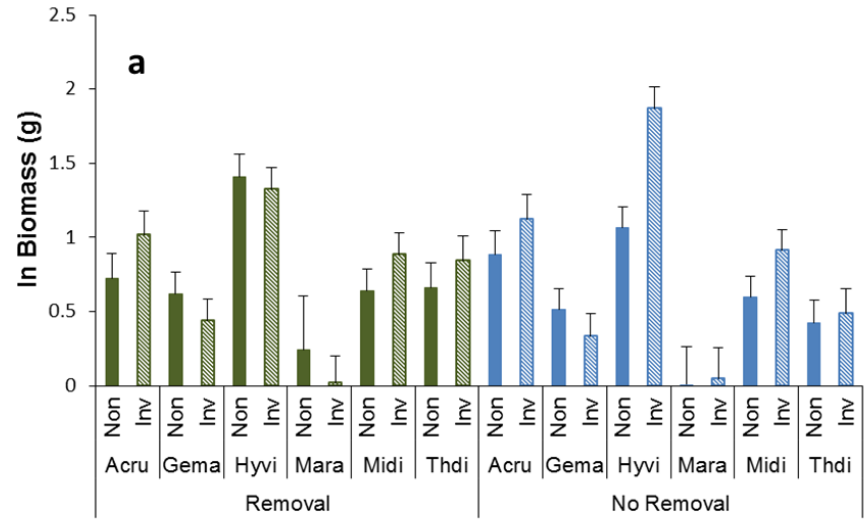


Figure 2-6. Biomass responses of three-year-old woodland herbs to garlic mustard invasion (invaded or non-invaded) and removal (full or no removal). **a)** WNC/CG species at CG; invasion by removal by species interaction was significant ($p = 0.05$); within no-removal treatments, Hyvi had significantly higher biomass in invaded plots compared to non-invaded plots ($p < 0.0001$); Hyvi also had significant negative responses to removal within invaded plots ($p = 0.009$). **b)** WNC/CG species at WNC; effects of invasion differed by species ($p = 0.02$); removal effects differed by species and light ($p = 0.01$); biomass was significantly higher in invaded plots for Thdi ($p = 0.01$) and nearly significant in Acru ($p = 0.06$); biomass was nearly significantly lower in invaded plots for Hyvi ($p = 0.07$). **c)** CG-only species. Both invasion and removal effects differed by species and light level ($p = 0.0008$ and 0.04 , respectively). Altr had consistently lower biomass in invaded plots; Phdi had consistently higher biomass in invaded plots; Syco had lower biomass in invaded plots at low light levels, but higher biomass in invaded plots at medium and high light levels (medium light levels shown). All three species had higher biomass in full removal plots at low light levels and lower biomass in removal plots at high light levels (medium light levels shown). Bars represent least squares means + 1 SE. Acru = *A. rubra*; Altr = *A. tricoccum*; Gema = *G. maculatum*; Hyvi = *H. virginianum*; Mara = *M. racemosum*; Midi = *M. diphylla*; Phdi = *P. divaricata*; Syco = *S. cordifolium*; Thdi = *T. dioicum*.

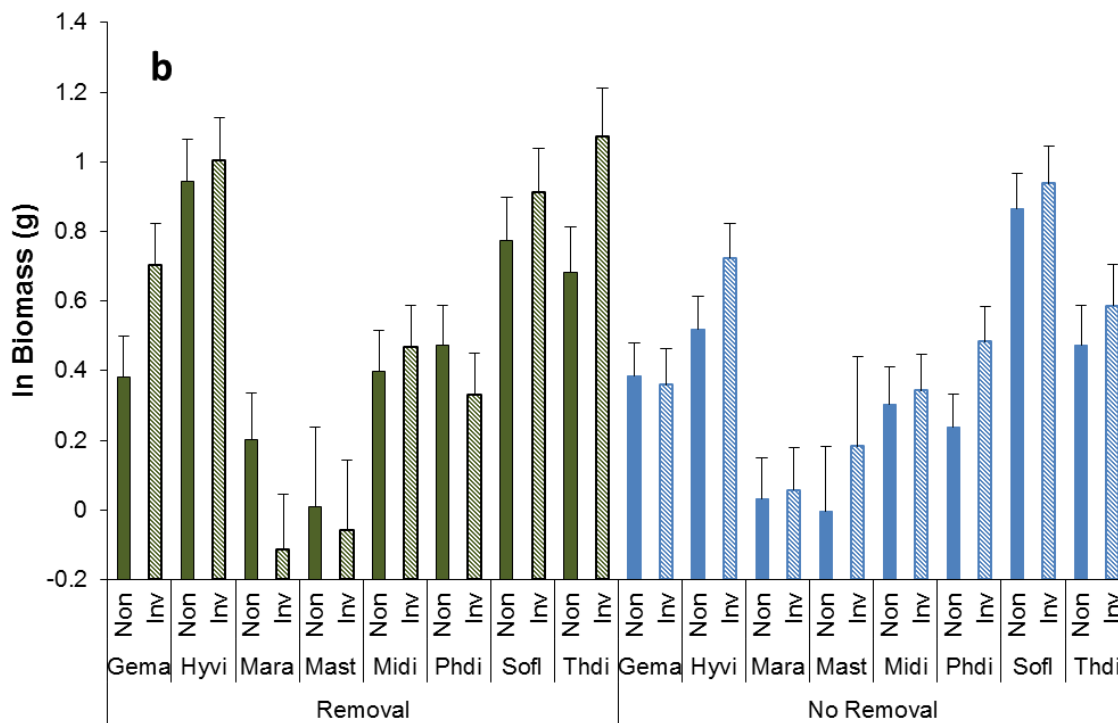
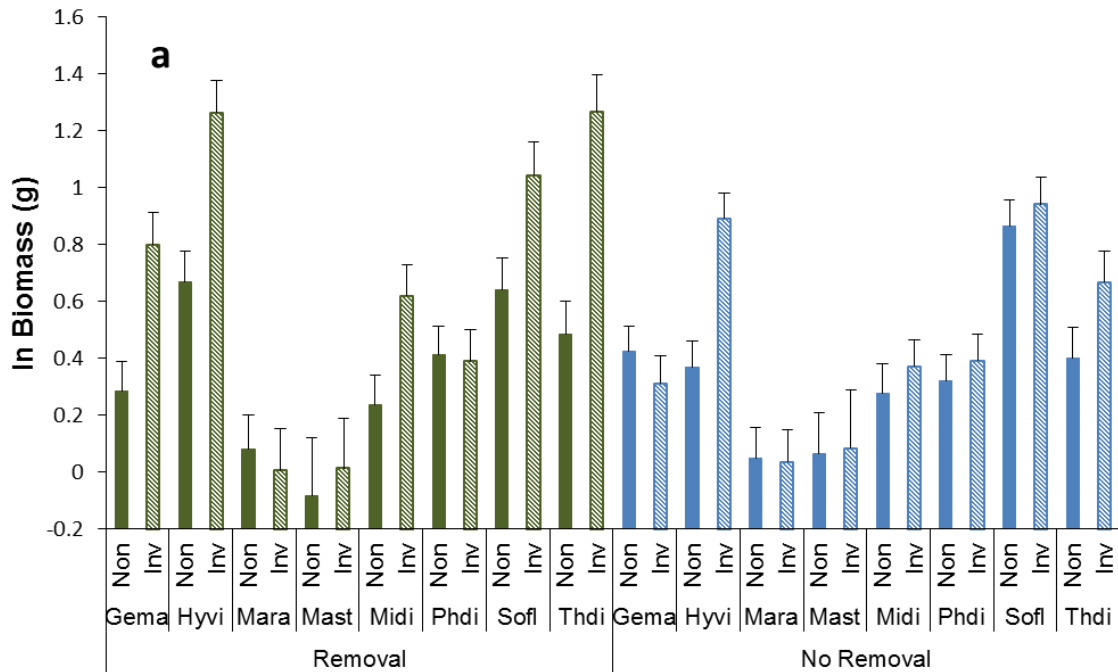


Figure 2-7. Biomass responses of two-year-old woodland herbs (WNC/CG species) to garlic mustard invasion at CG; **a)** results of analysis without nutrient covariate; invasion and removal effects differed by species ($p < 0.0001$ and $p = 0.02$, respectively); Gema,

Hyvi, Midi, Sofl and Thdi all had significantly higher biomass in invaded plots; the invasion by removal by species interaction was nearly significant ($p = 0.06$); **b**) results of analysis with nutrient covariate included to separate out the potential fertilization pathway from garlic mustard's other mechanisms of impact; invasion by removal by species interaction was significant ($p = 0.04$); vegetation removal had significant positive effects on Gema in invaded plots ($p = 0.03$) and Hyvi in non-invaded plots ($p = 0.004$). Bars represent least squares means + 1 SE. Gema = *G. maculatum*; Hyvi = *H. virginianum*; Mara = *M. racemosum*; Mast = *M. stellatum*; Midi = *M. diphylla*; Phdi = *P. divaricata*; Sofl = *S. flexicaulis*; Thdi = *T. dioicum*.

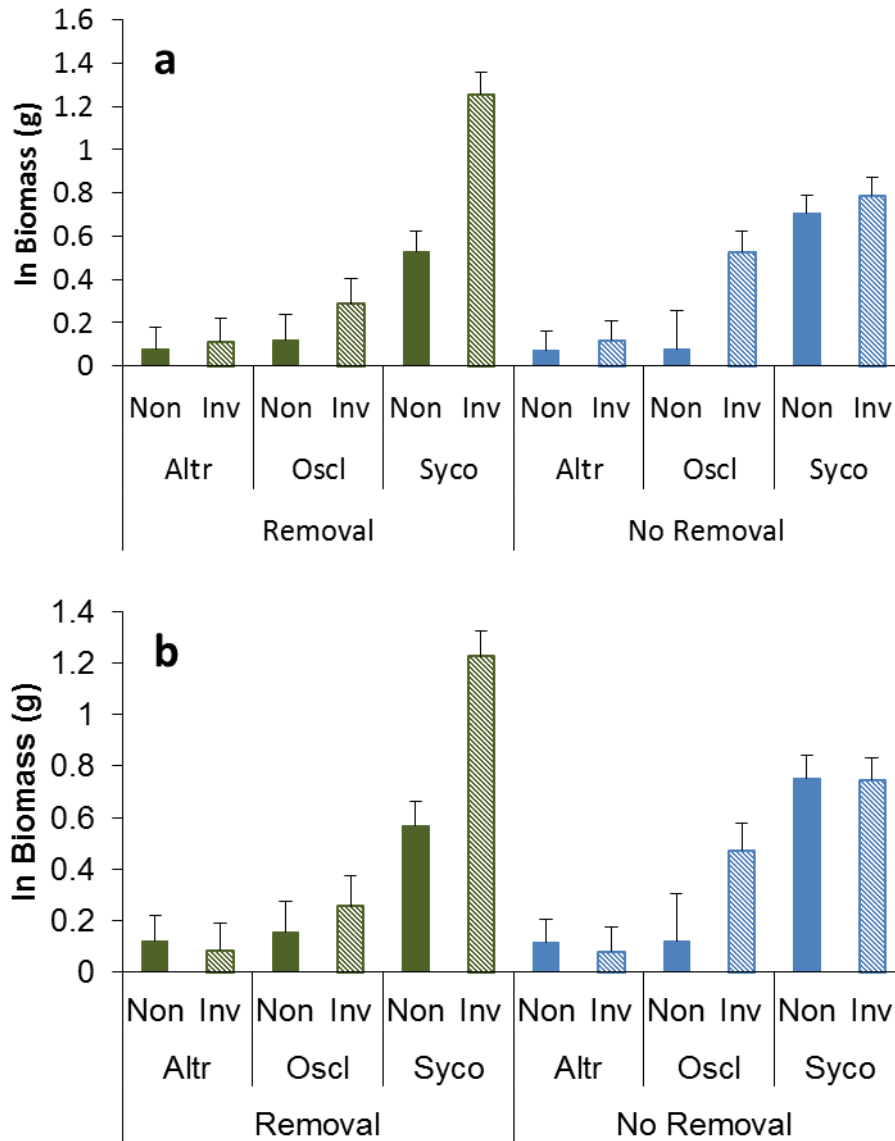


Figure 2-8. Biomass responses of two-year-old woodland herbs (CG-only species) to garlic mustard invasion and removal **a)** without nutrient covariate included in the model; the invasion by removal by species interaction was significant ($p = 0.002$); **b)** with nutrient covariate included; the invasion by removal by species interaction was significant ($p = 0.002$). In both analyses, within removal treatments, *S. cordifolium* had significantly higher biomass in invaded plots than non-invaded plots ($p < 0.0001$), as well as significant positive responses to garlic mustard removal ($p = 0.0003$). *O. claytonii* also had positive responses to invasion within no-removal plots, but this effect was only

significant in the analysis without the nutrient covariate ($p = 0.03$). Bars represent least squares means \pm 1 SE. Altr = *A. tricoccum*; Oscl = *O. claytonii*; Syco = *S. cordifolium*.

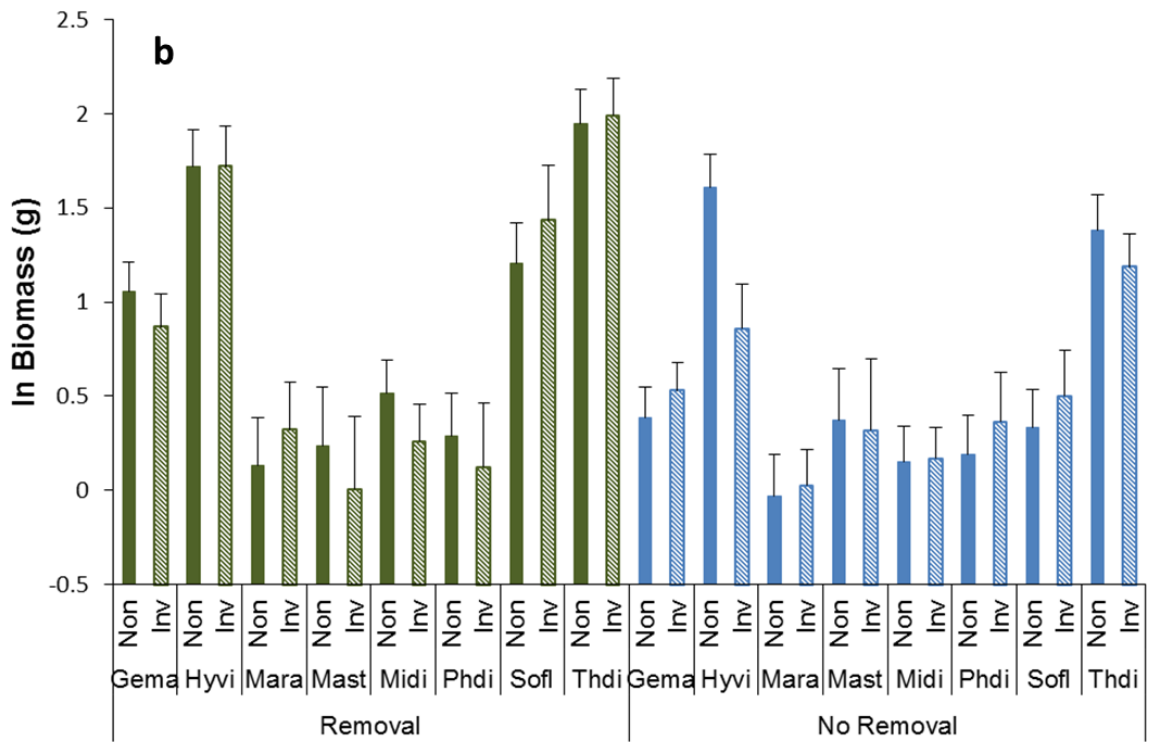
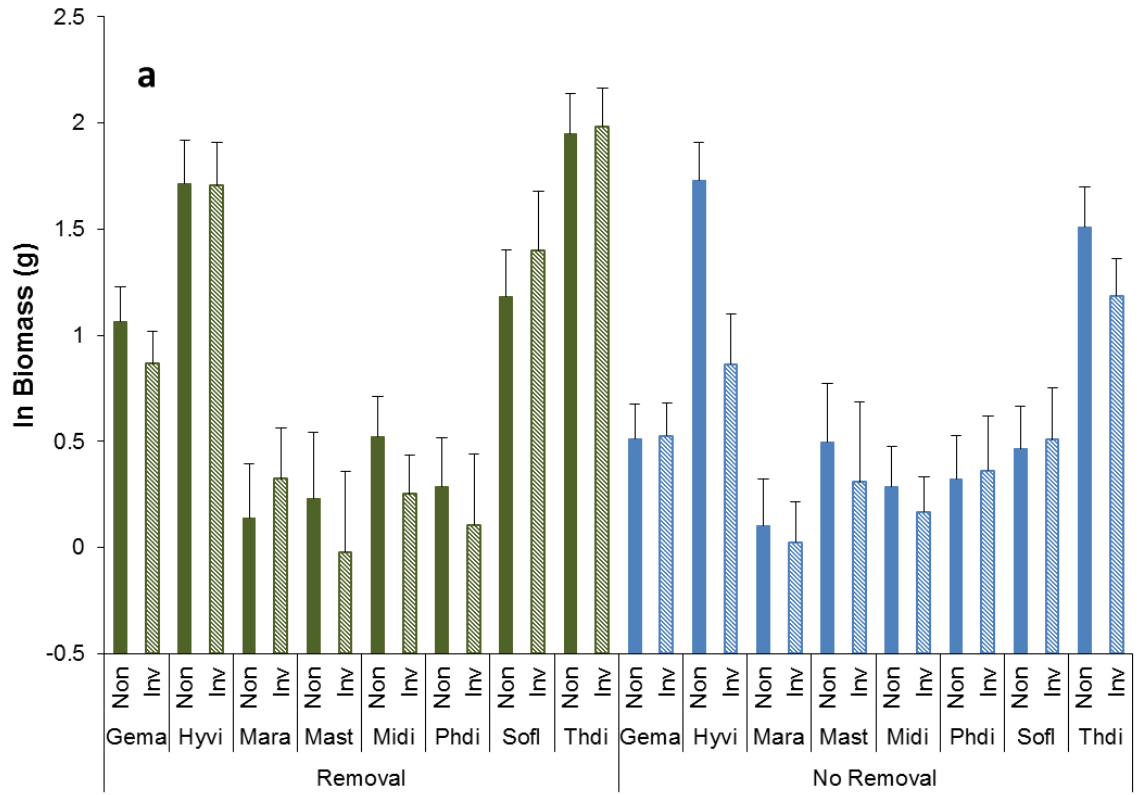
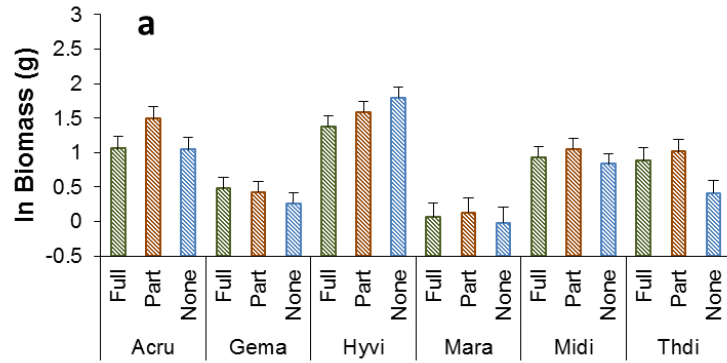
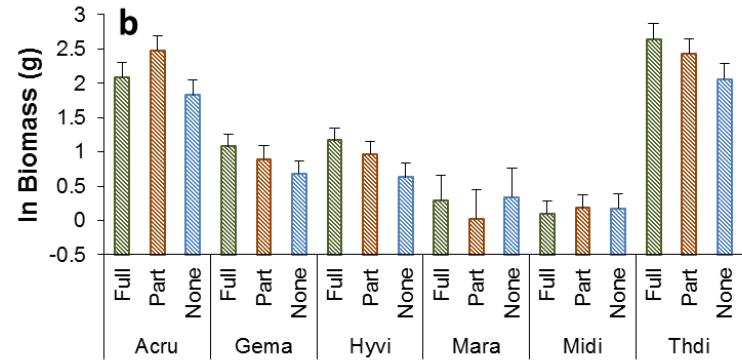


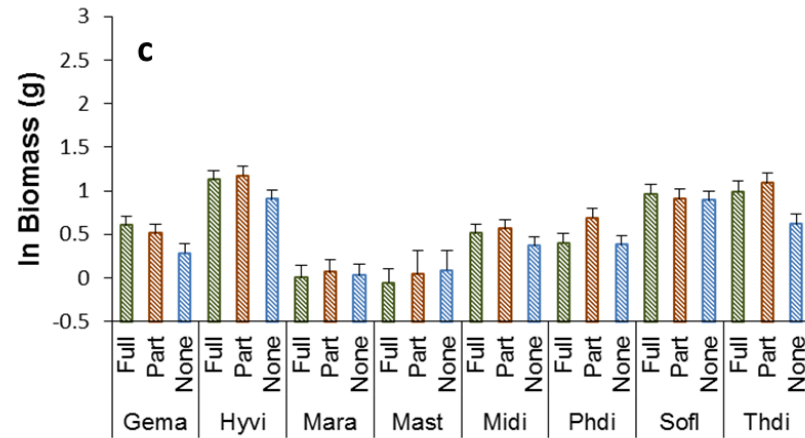
Figure 2-9. Biomass responses of two-year-old woodland herbs (WNC/CG species) to garlic mustard invasion at WNC; **a)** results of analysis without nutrient covariate; the invasion effect was not significant across species; removal effects varied by species and above-plot light level ($p = 0.01$); **b)** results of analysis with nutrient covariate included to separate out the potential fertilization pathway from garlic mustard's other mechanisms of impact; invasion effects interacted significantly with the nutrient covariate ($p = 0.05$); at low nutrient levels, biomass was lower in invaded plots, but at higher nutrient levels, biomass was higher in invaded plots. Removal effects differed by species and light level ($p = 0.009$). Bars represent least squares means ± 1 SE. Gema = *G. maculatum*; Hyvi = *H. virginianum*; Midi = *M. diphylla*; Mara = *M. racemosum*; Mast = *M. stellatum*; Phdi = *P. divaricata*; Sofl = *S. flexicaulis*; Thdi = *T. dioicum*.



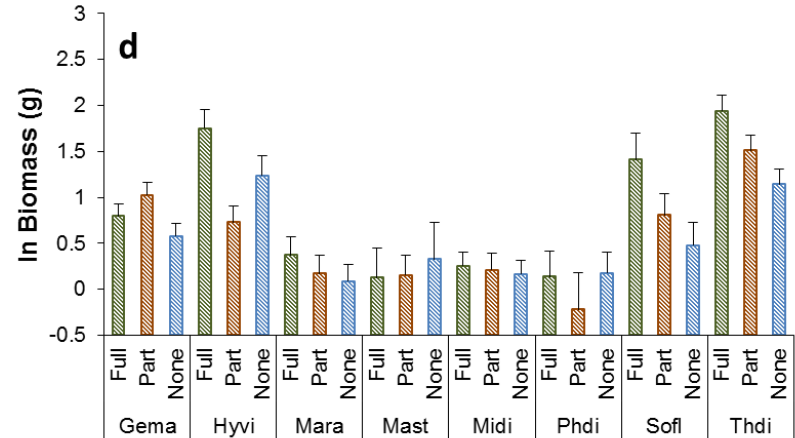
CG - 3-year-old herbs



WNC - 3-year-old herbs



CG - 2-year-old herbs



WNC - 2-year-old herbs

Figure 2-10. Biomass responses of woodland herbs to different degrees of garlic mustard removal (full, partial, and no removal); **a)** CG: three-year-old herbs in plots with three years of removal; biomass differed significantly by species ($p < 0.0001$) and light ($p = 0.03$), but not by removal; **b)** WNC: three-year-old herbs/three years of removal; biomass differed significantly by species ($p < 0.0001$) and light ($p < 0.0001$), but not by removal; **c)** CG: two-year-old herbs/two years of removal; biomass differed significantly by species ($p < 0.0001$) and light ($p < 0.02$), but not by removal; **d)** WNC two-year-old herbs/two years of removal at WNC; biomass differed significantly by light ($p = 0.0008$), and there was a significant removal by species interaction ($p = 0.02$); *S. flexicaulis* and *T. dioicum* had significantly higher biomass in full removal plots than in no-removal plots ($p = 0.01$ and 0.0009 , respectively); *G. maculatum* had significantly higher biomass in partial removal plots than in no removal plots ($p = 0.02$); and *H. virginianum* had significantly higher biomass in full removal compared to partial removal. Bars represent least squares means + 1 SE. Acru = *A. rubra*; Gema = *G. maculatum*; Hyvi = *H. virginianum*; Mara = *M. racemosum*; Mast = *M. stellatum*; Midi = *M. diphylla*; Phdi = *P. divaricata*; Sofl = *S. flexicaulis*; Thdi = *T. dioicum*.

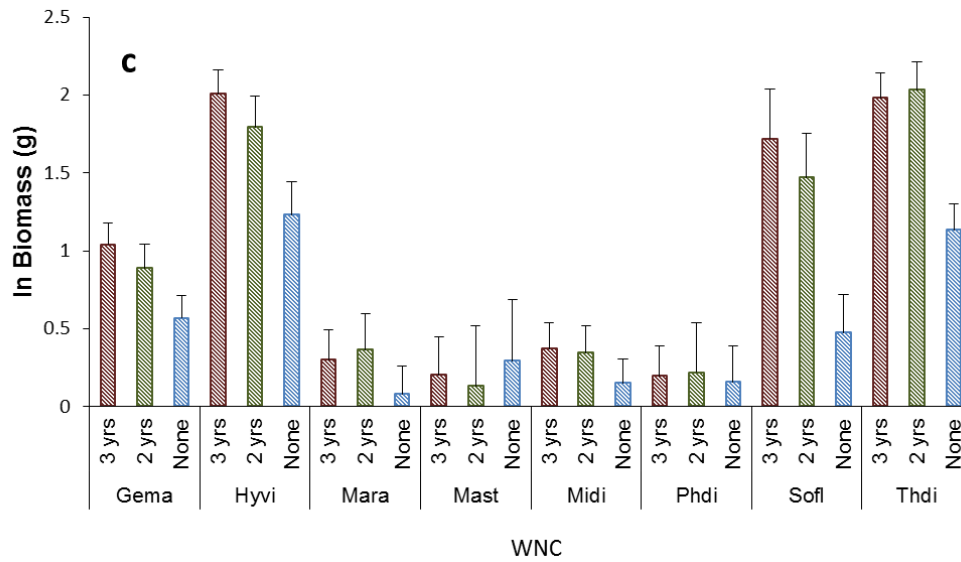
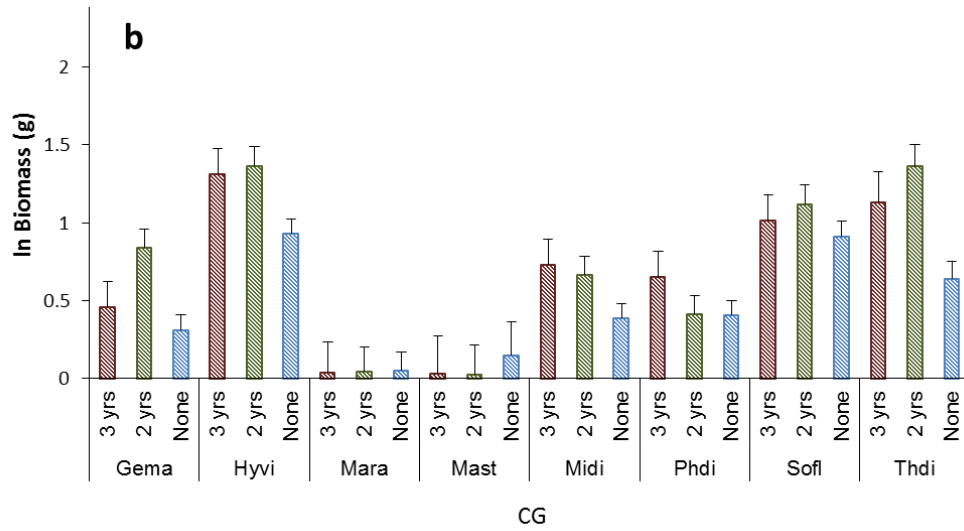
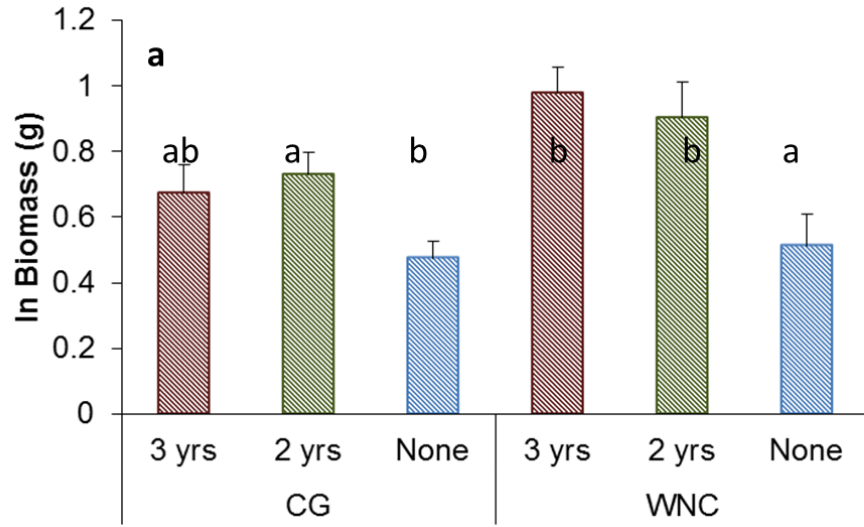


Figure 2-11. Biomass responses of woodland herbs to duration of garlic mustard removal (total of 3 years, 2 years or no removal at time of harvest). **a)** overall trends across species at two study sites (sites analyzed separately); letters indicate statistically significant differences ($p < 0.05$). At CG, removal effects differed by species ($p = 0.05$) and by nutrient level ($p = 0.04$; results at medium nutrient levels are shown); at WNC, removal effects were significant without treatment interactions ($p = 0.01$); both two and three years removal differ significantly from no-removal ($p = 0.02$ and 0.004 , respectively), but not from each other; **b)** 2 year old plants at CG; removal effects differed by herb species ($p = 0.05$); biomass was significantly lower in no removal vs. two years removal in Gema, Hyvi, and Thdi, and vs. three years removal in Hyvi and Thdi ($p < 0.05$); **c)** 2 year old plants at WNC. Bars represent least squares means + 1 SE. Gema = *G. maculatum*; Hyvi = *H. virginianum*; Mara = *M. racemosum*; Mast = *M. stellatum*; Midi = *M. diphylla*; Phdi = *P. divaricata*; Sofl = *S. flexicaulis*; Thdi = *T. dioicum*.

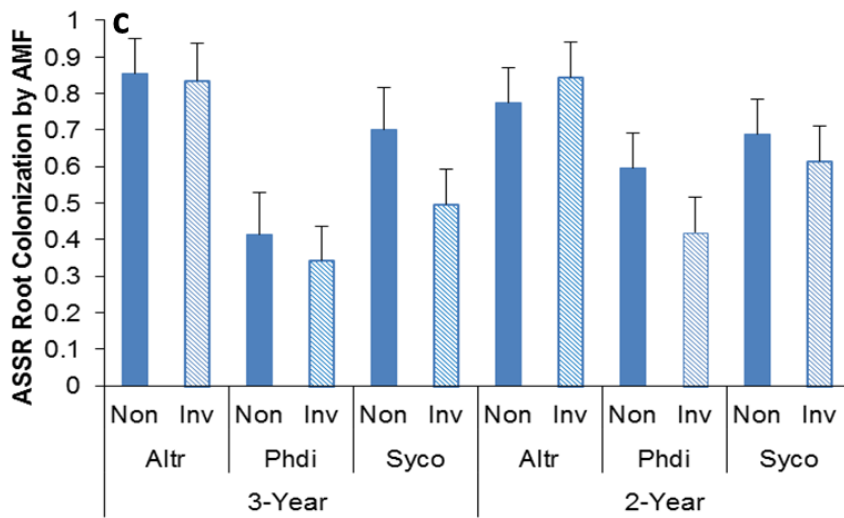
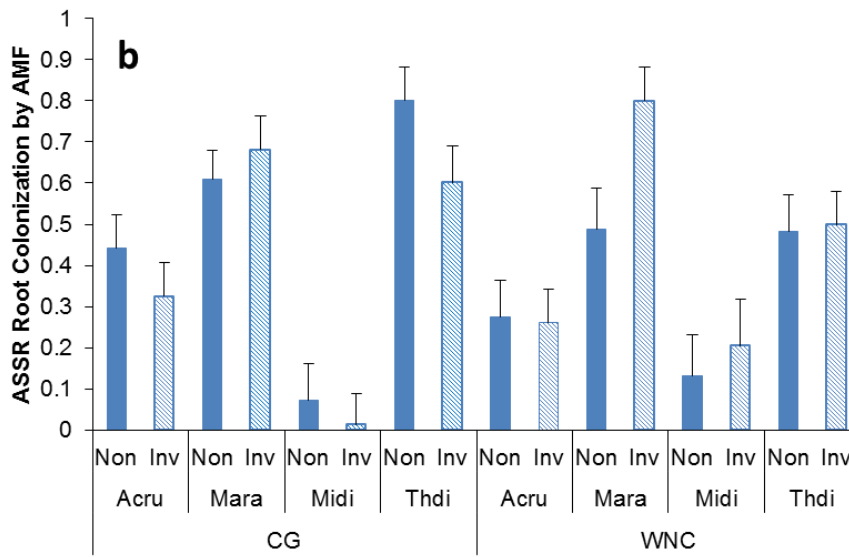
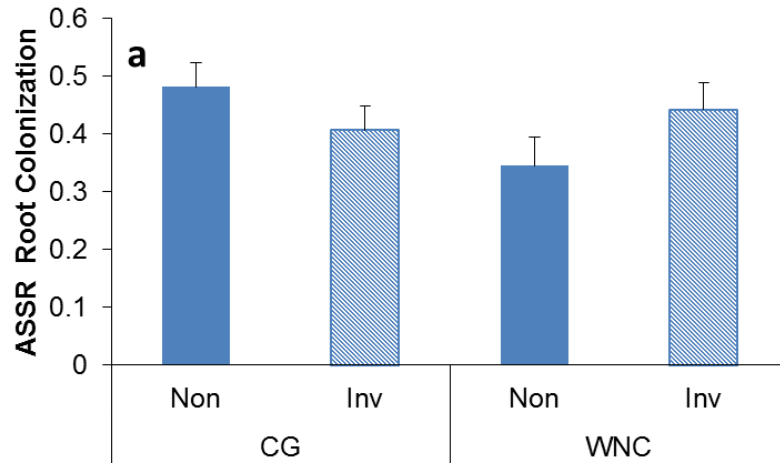


Figure 2-12. AMF root colonization in planted herbs. **a)** WNC/CG species – invasion by site trends across species ($p = 0.07$); **b)** WNC/CG species analyzed across study sites; AMF differed significantly by species ($p < 0.0001$); species by invasion and species by site interactions were nearly significant ($p = 0.08$ and 0.06 , respectively); **c)** CG-only species; comparison of three and two-year old plants; only the species main effect was significant in this analysis ($p < 0.0001$). Proportion of root colonized is ASSR-transformed. Bars represent least squares means + 1 SE.

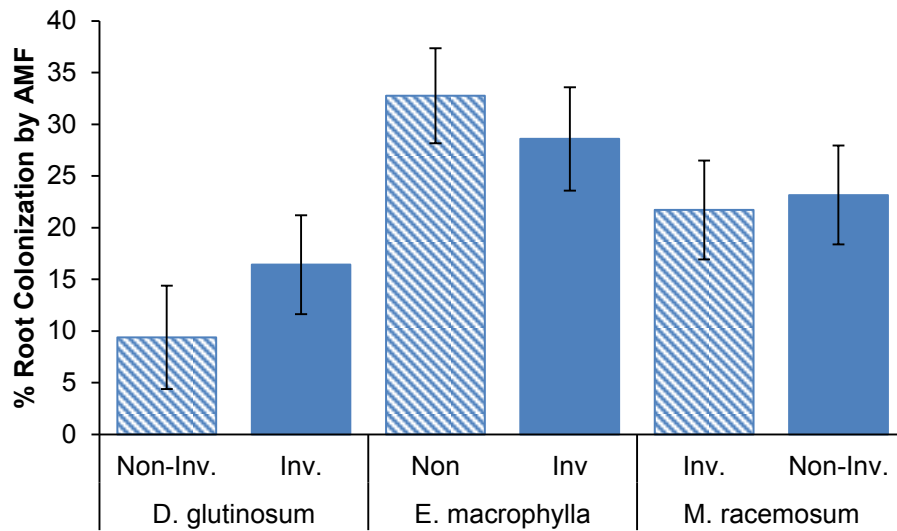


Figure 2-13. Mean percentage of root colonized by arbuscular mycorrhizal fungi (AMF) in herbs collected from within a garlic mustard-invaded and non-invaded area at Warner Nature Center, Marine-on-St. Croix, MN. AMF colonization differed significantly by plant species, but there were no significant differences between invaded and non-invaded areas across or within species. No evidence of AMF colonization was observed in *G. maculatum* and *A. petiolata*. Bars represent means \pm 1 SE. Letters indicate statistically significant differences by plant species ($p < 0.05$).

Chapter 3

Soil-Mediated Impacts of Garlic Mustard (*Alliaria petiolata*) on Germination, Seedling Establishment and Fungal Root Colonization of Woodland Herbs

Invasive plants that alter biotic, chemical or physical attributes of soil may have detrimental impacts on native plants and leave soil legacies that persist following invasion control. The invasive biennial herb garlic mustard appears to negatively affect woodland plant communities via its “novel weapons” of allelopathy, both direct and mediated through arbuscular mycorrhizal (AM) fungi. Attempts to assess garlic mustard’s soil-mediated impacts on woodland herbs have thus far yielded conflicting results. In this greenhouse study, I aimed to test garlic mustard’s soil-mediated impacts on a broader array of native woodland herbs in order to anticipate likely community responses to invasion and inform restoration strategies. I tested garlic mustard’s impacts on germination, growth (biomass) and AM- and non-AM fungal root colonization of 13 native herbs seeded into field soils collected from invaded and non-invaded areas. A fungicide treatment (Benomyl) was also included to explicitly test the AMF mechanism of impact. Herb seed germination was significantly lower in soils with a history of garlic mustard compared to non-invaded soils, and germination timing appeared to be accelerated in invaded soils, although the effect was not significant. Invaded soils and fungicide both moderately reduced AMF colonization, but the subsequent effects on biomass differed, possibly due to non-uniform impacts on fungal species. Biomass was consistently higher in the fungicide treatment but responses to invaded soils differed by experimental replication. Herb biomass was lower in invaded soils in the first experimental replication, when plants were inadvertently subjected to climate stress, but higher in invaded soils in the second replication, under controlled climate conditions. The inconsistent response suggests that garlic mustard’s negative impacts on herbs only

manifest when combined with additional stressors. Additionally, colonization rates of non-AM fungi were higher in invaded soils and in the first experimental replications; garlic mustard may accumulate pathogens, or by reducing AMF, make plants more vulnerable to infection by non-AM fungi under conditions of environmental stress. Garlic mustard may impact herb communities through multiple interacting soil-mediated pathways, and the net effect may depend on site conditions and the presence of additional stressors. Garlic mustard's negative effect on seed germination could adversely impact long-term population dynamics, as woodland herb regeneration and post-invasion recovery and restoration by seed may be inhibited.

Introduction

The rapid spread of the invasive herb garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] in North American hardwood forests is a great concern to managers tasked with conserving already-stressed native woodland plant communities. Garlic mustard invasion is often implicated in the degradation of woodland communities, with reports of simultaneous declines in native species and inverse relationships between garlic mustard and native plants cited as evidence (Nuzzo 1991, Van Riper et al. 2010). The threat garlic mustard may pose to North American woodlands is considered sufficient to warrant development of a biocontrol program to reduce its invasion and spread in North America (Skinner and Blossey 2005, Evans and Landis 2007, Van Riper et al. 2010). Multiple mechanisms appear to contribute to garlic mustard's invasive success and impacts on woodland communities (Rodgers et al. 2008a), however its potential for direct allelopathy and disruption of plant-fungal mutualisms has received considerable attention, in part due to current interest in the "novel weapons hypothesis" of plant invasion (Callaway and Ridenour 2004, Callaway et al. 2008, Barto et al. 2010a), but also because it suggests serious management implications: by altering soil chemistry and biota, garlic mustard may leave a legacy that continues to affect native plants even after garlic mustard is effectively controlled.

Like many members of the Brassicaceae family, garlic mustard's leaf and root tissue extracts contain glucosinolates (i.e. sinigrin) and secondary metabolites that are known to have allelopathic and antifungal properties (Vaughn and Berhow 1999, Fahey et al. 2001). Although allelopathy is recognized as a potential pathway of impact in invaded plant communities (Inderjit and van der Putten 2010), investigations of garlic mustard's direct allelopathic effects on crop species and native plants have yielded conflicting results, depending on the species tested, extracts and application levels used and other experimental conditions (McCarthy and Hanson 1998, Cipollini et al. 2008a, Cipollini et al. 2008b, Barto and Cipollini 2009a, Lankau 2010, Pisula and Meiners 2010). Native species tested have exhibited moderate reductions in germination and/or growth (Prati and Bossdorf 2004, Barto et al. 2010b), though in some cases, only at extract levels that exceed those found in the field (Barto and Cipollini 2009a). Moreover, relatively few native plant species (primarily annuals) have been studied in this manner, so it is unknown how common sensitivity to garlic mustard's allelopathy is among woodland plants.

Similarly, while the anti-fungal properties of garlic mustard extracts have been demonstrated, resulting in reduced AMF sporulation, levels in soils, and colonization in some plant roots (Roberts and Anderson 2001, Stinson et al. 2006, Callaway et al. 2008, Anderson et al. 2010, Cantor et al. 2011), the impacts on native plants, particularly herbs, are not entirely clear. An estimated 80% of terrestrial plant species support associations with AMF (Wang and Qiu 2006), a relationship that is generally considered symbiotic: in exchange for photosynthates (carbon), AMF hyphae can increase a plant's access to limited soil resources, particularly phosphorus (Siqueira and Saggin-Júnior 2001, Smith et al. 2003b, Jia et al. 2004), nitrogen (Barea et al. 1987, George et al. 1992) and water (George et al. 1992, Augé 2001), resulting in increased survival and growth (Berta et al. 1995). However, the degree to which plants benefit from mycorrhizal associations varies by species (Wilson and Hartnett 1998, Siqueira and Saggin-Júnior 2001), which suggests the AMF-mediated impacts of garlic mustard may vary as well. Garlic mustard has, in fact, been shown to negatively affect tree seedling growth by reducing levels of AMF

colonization of roots (Stinson et al. 2006), but studies of herbs have been less conclusive. Callaway et al. (2008) found that garlic mustard extracts strongly inhibited mycorrhizal colonization of North American herbaceous plants, resulting in reduced seedling emergence, survival and growth. However, other studies have reported no reduction in AMF colonization (Burke 2008), or minimal impact on the colonized herbs (Koch et al. 2011). Stinson et al. (2006) found that herbs were less dependent on AMF and less affected by garlic mustard presence than were woody species; however the herbs included in their study were generally disturbance-adapted “edge” species as opposed to perennial herbs typical of woodland interior. Although the majority of deciduous woodland herbs examined have been found to form mycorrhizal associations (McDougall and Liebtag 1928, Brundrett and Kendrick 1988, Berliner and Torrey 1989, DeMars 1996, Whigham 2004), relatively few herbaceous woodland perennials have been tested for sensitivity to garlic mustard’s soil-mediated effects, and the overall community-level effects of garlic mustard remain unclear.

In this study, I examined the effects of garlic mustard on the seeds and seedlings of 13 native woodland herbs, representing 12 different plant families (Table 1). In two separate greenhouse experiments, I evaluated garlic mustard’s effects on germination, establishment, and fungal colonization of roots. The seedling germination study compared the germination rates of native seeds planted into field-collected soils from invaded and non-invaded areas. In the seedling establishment study, I compared native seedling growth (biomass) and AM- and non-AM fungal root colonization in a factorial design with invasion history and fungicide application treatments.

I hypothesized that if garlic mustard negatively impacts native seedling establishment, then seeds planted into soils from garlic mustard-invaded areas (hereafter: “invaded soils”) would have lower rates of germination and produce smaller seedlings than those planted into soils from non-invaded areas (hereafter: “non-invaded soils”; Figure 1). Additionally, if the primary mechanism by which garlic mustard affects native herbs is suppression of AMF, then seedlings growing in invaded soils would have

lower percent root colonization by AMF than those growing in non-invaded soils; their growth patterns would be comparable to those of seedlings treated with fungicide; and fungicide would have a greater effect on seedlings growing in non-invaded soils. I also expected that seedlings growing in the low-AMF treatments (invaded soils and fungicide-treated) would invest a greater fraction of their total biomass to roots (Berta et al. 1995) in order to access limited soil resources. The interaction of invasion history by fungicide treatments was predicted because, presumably, AMF in invaded soils would already be suppressed by garlic mustard, and therefore plant responses to the fungicide treatments would be minimal. Finally, by comparing the effects of garlic mustard invasion history and fungicide application on non-AM fungal colonization, I tested the assumptions that both garlic mustard and the selected fungicide (Benomyl) primarily affect AMF. Although research on garlic mustard's antifungal properties have largely focused on AMF, glucosinolates and their byproducts isolated from other Brassica species have been shown to suppress other fungal species, including common plant pathogens (Manici et al. 1997); non-AM fungi may therefore represent another indirect pathway of impact on native herbs.

A primary objective of this study was to assess garlic mustard's soil-mediated impacts across a broader range of native species than has been previously examined, as well as to expand the geographic range of garlic mustard impact studies, identified as a future research priority in invasion studies (Wolfe and Klironomos 2005). Additionally, I aimed to gain further insights into the nature of plant-fungal relationships in native woodland herbs, as our understanding of these associations is still quite limited. I focused on impacts on seeds and seedlings, because this vulnerable life stage is particularly sensitive to environmental stress, including allelopathy and soil biotic interactions (Janos 1980, Van der Putten 2003, Fenner and Thompson 2005), and because reproduction by seed is critical to the ability of plant populations to adapt to environmental change (Davis et al. 2005, Fenner and Thompson 2005, Aitken et al. 2008). The emphasis on herbaceous plants was motivated partly by the inconsistent effects reported previously, and the relatively small number of species examined, but also

because of the important conservation and restoration implications of garlic mustard's potential impacts on the herb layer. Woodland herbaceous communities can include more than 80% of the total plant species richness of forests (Gilliam 2007) strongly influencing ecosystem dynamics, including nutrient cycling, soil biotic activity, tree species regeneration, and successional trajectories (Muller 2003, Nilsson and Wardle 2005, Gilliam 2007). However, declines in herb diversity and abundance have been widely observed (Brewer 1980, Robinson et al. 1994, Rooney et al. 2004, Rooney and Rogers 2011), driven by a variety of anthropogenic and ecological stressors, including over-harvesting (Jolls 2003); logging (Duffy and Meier 1992, Meier et al. 1995, Small and McCarthy 2002), agriculture (Singleton et al. 2001, Flinn and Vellend 2005), urbanization (Drayton and Primack 1996); herbivory pressure from white-tailed deer (*Odocoileus virginianus* Zimmerman) (Rooney and Waller 2003, Côté et al. 2004, Webster et al. 2005, Wiegmann and Waller 2006) and slugs (Hahn et al. 2011); invasive plants (Woods 1993, Gould and Gorchoff 2000, Collier et al. 2002, Frappier et al. 2003, Greene and Blossey 2011) and earthworms (Bohlen et al. 2004, Frelich et al. 2006, Nuzzo et al. 2009). Given the numerous threats to herb communities, it is important to understand the extent to which invasive plants such as garlic mustard may contribute to their decline, so that conservation management activities can be prioritized. Furthermore, planted communities of native herbs have been shown to inhibit garlic mustard invasion (Chapter 4); a more comprehensive understanding of how species differ in their tolerance and sensitivity to garlic mustard's impacts can inform both predictions of future shifts in community composition, i.e. "winners and losers" (McKinney and Lockwood 1999, Wiegmann and Waller 2006), as well as species selection for post-invasion restoration (Perry et al. 2005, Jordan et al. 2008).

Methods

Soil Characterization and Collection

In October 2008, I collected 150 liters of field soil (10 cm depth) from a dense garlic mustard population and a nearby non-invaded native community (distance between soil collection sites approximately 100 m) within a dry-mesic oak forest at Warner Nature Center in Marine-on-St. Croix, Minnesota, 35 km northeast of the city of Saint Paul. The soil in the study area is Kingsley sandy loam (Soil Survey Staff 2010), and analysis by hydrometer method (Day 1965) for another study (Chapter 2) found no significant differences in soil texture between the invaded and non-invaded areas at this site (mean percentages of sand, clay and silt in the invaded and non-invaded areas were, respectively: 72%, 5%, and 23%, and 75%, 3%, and 22%). A bulked subsample from each soil treatment (invaded and non-invaded) was sent to the University of Minnesota Soil Testing Laboratory (St. Paul, MN) to characterize the soil chemistry of the two collection areas. Invaded soil had slightly higher pH than non-invaded soil, and had slightly to moderately higher levels of organic matter, nitrate, potassium, calcium, and magnesium, but phosphorus levels were very high in both invaded (49 ppm) and non-invaded (50 ppm) soils (Table 2). This characterization was consistent with a comparison of soil nutrient availability conducted for another study (Chapter 2), which found that both nitrogen and phosphorus availability was significantly higher in invaded areas than non-invaded areas at this site.

Soils were bulked by invasion history (invaded vs. non-invaded) and spread onto plastic sheets on greenhouse benches to air dry for 2 weeks. During this time, I manually mixed the soil, removed rocks, roots and litter, and broke up large soil aggregates. The soil was then bagged and placed in cold storage (4 °C) until used. Field soils were not sterilized, leaving their biotic component (including, but not limited to, AMF) active.

Seed Species and Sources

Native seeds were purchased in November 2008 from Prairie Moon Nursery (Winona, MN), with the exception of two species, *Geranium maculatum* and *Allium tricoccum*, that were purchased in Sept. 2007. Seeds were stored at 2 °C until stratified or planted (Table 1), as recommended by Prairie Moon Nursery, Winona, MN and Cullina (2000). The species selected were typical of dry-mesic oak forests in Minnesota and represented a range of plant families, life history characteristics, and expected mycorrhizal status (McDougall and Liebttag 1928, Boerner 1986, Newman and Reddell 1987, Brundrett and Kendrick 1988, DeMars 1996, Miller et al. 1999). Taxonomy follows USDA, NRCS (2011).

Experiment 1: Germination

In this experiment, I tested the effects of garlic mustard-invaded soils on germination of 12 native plant species. For each species, I divided equal numbers of seeds into 10 samples and stratified as appropriate (Table 1). For two species with seeds that were too small to reasonably count (*Scrophularia marilandica* and *Symphyotrichum cordifolium*), I weighed equal quantities of seed on a balance, and then used average seed weight to estimate the number of seeds per sample.

In July 2009, I planted the seeds into 12 x 12 x 6 cm square plastic pots filled with a 1:1:2 mixture of field soil (invaded or non-invaded; effectively a 25% inoculum rate), Sunshine LC8 Professional Growing Mix potting soil, and steam-sterilized sand to minimize compaction and potential nutrient differences between treatments. I covered the seeds with a thin layer of vermiculate: 0.1 cm for light-sensitive seeds (*Scrophularia marilandica* and *Solidago flexicaulis*), and 0.5 cm for all other species.

For each species, there were five replicate pots per soil type (invaded and non-invaded). Pots were arranged in seedling trays according to invasion treatment to avoid soil contamination, and trays were placed in a greenhouse under natural light conditions and temperature maintained at an average of 24 °C (Figure 2). I periodically rearranged

the trays on the greenhouse bench to minimize location effects, and I watered the trays daily, or as needed to prevent desiccation.

Trays were checked for germination 1-2 times per week for 12 weeks (until germination ceased). Weeds were removed and discarded, and planted seedlings were counted and removed upon identification. At the end of the study, germination was summed for each pot and the percent germination was calculated. Data were arcsine-square root transformed to improve normality. I analyzed the differences in percent germination by soil type and species with ANOVA (proc glm; SAS 9.2). Effects of soil history and species on germination timing were also assessed. The number of days to peak (100%), 75%, and 50% germination were calculated for each experimental pot and analyzed with ANOVA (proc glm; SAS 9.2). Data were natural log transformed to improve normality.

Experiment 2: Seedling Establishment

In this experiment, I tested the hypothesis that garlic mustard-invaded soils would have a negative effect on seedling growth and levels of AMF colonization in roots. In a full-factorial design, I planted seeds of native species into pots of invaded and non-invaded field-collected soils, half of which were treated benomyl, a fungicide that has been used extensively in mycorrhizal studies and is found to effectively reduce levels of AMF colonization in plants (Hartnett and Wilson 1999, Smith et al. 2000, Callaway et al. 2004, Jordan and Huerd 2008). I conducted two replicates of this experiment: the first was planted in February and harvested in June 2009 (15 weeks), and the second was planted in July, and harvested in September 2009 (12 weeks). For each of 11 native species (Table 1), 96 “cone-tainer” pots were arranged in a rack and randomly assigned to a soil invasion treatment and a fungicide treatment (24 replicates per species of each treatment combination). The pots were filled with approximately 120 ml of a 1:1:2 mixture of field soil (invaded or non-invaded; 25% inoculum rate), potting soil, and steam-sterilized sand (as described in the germination study). I planted the seeds by spreading them on the soil surface of each pot, visually estimating an approximately even

numbers of seeds per species per pot, and then covered the seeds with a thin layer of vermiculate, as in the germination study.

I began applying fungicide treatments approximately two weeks following seedling germination, and continued them weekly until seedling harvest. I applied 5 ml of a solution of 0.58 g benomyl per 1 L of water to each treated pot, for an effective application rate of 2.5 g/m². All pots were lightly watered following fungicide application, in order to move the fungicide through the soil column, and to minimize soil moisture differences due to the fungicide application.

All pots were watered daily (or as needed) using the mist-setting of a hose nozzle to avoid contamination between soil treatments. Pots drained freely and did not share a water reserve. Overhead lights were on in the greenhouse to compensate for shorter day-length during the first 7 weeks of Replicate 1, but only natural lighting was used for the remaining weeks of Replicate 1 and the duration of Replicate 2. Temperature controls were set at 24 °C, although a malfunction caused temperatures to climb in May and June (Figure 2). To impose nutrient-limited conditions and increase potential seedling dependence on AMF, no fertilizer was added to the soils through the duration of the experiment.

After seedlings formed their first true leaves, they were thinned to one per pot. At the end of the experiment, I harvested the plants and washed the roots over a screen to remove soil particles. Harvested plants were dried for 7-10 days at 60 °C. Dried above- and below-ground biomass were weighed separately and used to calculate total plant biomass and root fraction (root biomass/total plant biomass). Germination rates and percent survival were not assessed in this experiment; only species that survived through the end of the experiment were harvested and included in the analysis.

Root Colonization by AM- and Non-AM Fungi

I selected three plant species to assess the response of root colonization by AMF to garlic mustard invasion history and fungicide application. Species selection was based in part on biomass responses, but was limited to those species that produced sufficient root mass to reasonably stain and view. Expected mycorrhizal status was also taken into account: *Symphyotrichum cordifolium* and *Solidago flexicaulis* were expected to be good mycorrhizal hosts, while *Carex brevior* was selected because it was thought to be non-mycorrhizal but showed unexpected biomass responses to invasion and fungicide.

Ten plants per forb species and five plants of *Carex brevior* from each invasion by fungicide treatment combination and experimental replication were randomly selected for root analysis for a total of 200 root samples. In preparation for AMF visualization, dried roots were cleared by autoclaving in KOH, rinsed with distilled water, acidified with HCl, and stained in aniline blue (Grace and Stribley 1991). I arranged a representative sub-sample of fine roots on a slide and viewed the roots at 200x magnification with a bright field microscope. Colonization was quantified by systematically viewing the slide and recording the presence or absence of AMF structures in each field of view (McGonigle et al. 1990). I recorded AMF as present if there were AMF vesicles, arbuscules, or hyphae visible within the root (Smith and Read 2008). Spores and “loose” hyphae (not penetrating the root) were not considered evidence of AMF colonization. Non-AM fungi, distinguished from AMF by the presence of septate or melanized hyphae, lack of typical AMF hyphal morphology (e.g. coiling), and attachment to non-AMF structures (Steinberg and Rillig 2003, Callaway et al. 2004, Smith and Read 2008), were also quantified in the same manner, although no attempt was made to identify them or assess potential pathogenicity. I calculated the percentage of root colonized by AMF and non-AM fungi separately by dividing the presence by the total number of views and multiplying by 100.

Statistical Analysis of Seedling Establishment and Root Colonization

I analyzed the effects of soil, fungicide and species on total plant biomass, root:shoot ratio, and AMF and non-AMF fungal colonization for each replicate of the experiment with a three-way ANOVA (proc glm; SAS 9.2). Above- and belowground biomass and root:shoot ratio were also analyzed, but the results did not differ substantially from those of total biomass and root fraction; therefore they are not included in this report. Type III sums of squares were used, because variable germination rates and survival across species and treatments resulted in an imbalanced design. With the exception of Table 1, all reported means are least squares means.

Results

Experiment 1: Germination

Eight of the 12 species germinated during the course of the experiment and were included in the analysis (Table 1). Germination rates differed significantly by species ($p < 0.001$) and by invasion history ($p = 0.03$), with six of the eight species having reduced germination in invaded soils (Figure 3a). The remaining two species, *Carex brevior* and *Solidago flexicaulis*, had higher germination rates in invaded soils, but the invasion by species interaction was not significant.

Germination timing differed significantly by species ($p < 0.001$), and the number of days to reach maximum germination (hereafter D_{100}) was slightly lower in invaded soil than non-invaded soil ($p = 0.09$; Figure 3b). The species by soil interaction was not significant. In all species, the majority of seedlings emerged fairly synchronously (within one week), with a few residual seedlings emerging in subsequent weeks. Soil invasion appeared to slightly decrease the number of days to reach 75% germination (D_{75}), but the effect was not significant. Soil invasion had no effect on the number of days to reach 50% germination (D_{50}).

Experiment 2: Seedling Establishment

Seven species had sufficient establishment in both replications of the experiment to be included in the analysis. *Thalictrum dioicum* germinated poorly and only in Rep. 1 and was therefore excluded (exclusion of this species did not affect the overall results of the Rep. 1 analysis).

Effects of garlic mustard invasion on seedling biomass differed by experimental replication (Figure 4a and b). Total biomass was significantly lower in invaded soils compared to non-invaded soils in the first replicate of the experiment ($p = 0.03$; Figures 4c). However, in Rep. 2, the opposite pattern was observed, with all species except *Solidago flexicaulis* having higher biomass in invaded soils (invasion by species interaction: $p = 0.02$; Figures 4d). Fungicide had an overall positive effect on seedling biomass in both experimental replications, though the effect differed by species (fungicide by species interaction: $p = 0.006$ and $p = 0.04$ in Reps. 1 and 2, respectively; Figure 4e and f). Seedling biomass was higher in the fungicide-treated soils than in untreated soils in all species, except for *Mitella diphylla*, which had lower biomass in fungicide-treated soils in both reps, though not significantly.

Fungicide appeared to reduce the effects of invasion when compared across all species (Figure 4a and b). Within the fungicide-treated soils, invasion had no significant effect in either replication, but within the untreated soils, invasion had a significant negative effect in Rep. 1 ($p = 0.008$), and a significant positive effect in Rep. 2 ($p = 0.002$). The effects of invasion on responses to fungicide treatment differed by experimental replication. In Rep. 1, fungicide had a significant positive effect regardless of invasion treatment ($p < 0.0001$ in invaded and $p = 0.003$ in non-invaded; Figure 4e), while in Rep. 2 the effect was only significant in the non-invaded soils ($p = 0.001$; Figure 4f). Despite the appearance of an invasion by fungicide interaction, this interaction was not statistically significant; however, there was a trend toward a three-way interaction between invasion, fungicide and soil in Rep. 1 ($p = 0.07$).

Analyses of above and belowground biomass individually mirrored that of total biomass in terms of overall patterns and direction of effects (data not shown). Patterns in aboveground biomass (shoots) differed from total biomass only in that in Rep. 2, the fungicide main effect was significant ($p = 0.003$), but not the fungicide by species interaction ($p = 0.08$). Like total biomass, belowground biomass (roots) showed a significant fungicide by species interaction, with overall higher biomass in fungicide-treated soils in both experimental replications, but invasion had a significant effect only in Rep. 2 (main effect: $p = 0.03$; soil by species interaction: $p = 0.06$; soil by fungicide interaction: $p = 0.07$).

Allocation of biomass to roots (i.e. root fraction) was affected by both invasion and fungicide treatment, particularly in the first experimental replication (Figure 5a and b). However, there was no evidence of an invasion by fungicide interaction in either replication. Overall, root fraction was higher in invaded soils (Figure 5c and d) and lower in fungicide-treated soils (Figure 5e) compared to non-invaded and untreated soils. In Rep. 1, the effects of both treatments differed by species, with significant soil by species ($p < 0.0001$) and fungicide by species ($p = 0.0001$) interactions. In Rep. 2 only the main effects of soil and species were significant ($p = 0.02$ and $p < 0.0001$, respectively). No within-species differences were significant in Rep. 2, however.

Analysis of Fungal Root Colonization in Native Seedlings

Ten root samples were analyzed from each experimental treatment and replication for three native species: *Carex brevior*, *Solidago flexicaulis*, and *Symphyotrichum cordifolium*. There was no evidence of AMF colonization in any of the *C. brevior* root samples, therefore the species was excluded from the AMF analysis. In the first experimental replication, AMF colonization rates were significantly lower in both invaded and fungicide-treated soils ($p = 0.0001$ and $p = 0.02$, respectively); no treatment interactions were significant, nor were there significant differences in colonization rates by plant species (Figure 6 a, c). In rep. 2, however, effects of invasion differed by species. As in Rep. 1, invaded soils had a significant negative effect on AMF

colonization rates of *S. cordifolium*, but there was no effect on colonization of *S. flexicaulis* (invasion by species interaction: $p = 0.006$). Fungicide had no significant effect on colonization by AMF in either species in Rep. 2. Although the effects of fungicide appeared more pronounced in invaded soil in Rep. 1 and in non-invaded soil in Rep. 2, the fungicide by invasion interaction was not significant in either replication.

Root colonization by non-AM fungi was highly variable. In Rep. 2, the statistical model did not fit the data unless one extreme value (a *Solidago flexicaulis* root sample from non-invaded, fungicide -treated soils with 42% root colonization) was removed. Although there is no reason (other than its extreme value) to believe this apparent outlier is erroneous, it was excluded from the analysis in order to fit the model to the remaining data. The results of Rep. 2 should therefore be interpreted cautiously.

Patterns of non-AM fungal colonization of roots differed by experimental replication. Overall, colonization rates were much higher in Rep. 1 than in Rep. 2, although this pattern was driven largely by high colonization rates in the invaded soils in Rep. 1 (Figure 7). In Rep. 1, there was a significant invasion by species interaction ($p < 0.0001$), with significantly higher colonization rates in garlic mustard-invaded soil in *Carex brevior* and *Solidago flexicaulis*, but only a marginal (non-significant) increase in *Symphyotrichum cordifolium*. In Rep. 2, however, the effects of invasion were not significant. Fungicide, however, significantly reduced non-AM colonization in the second rep. ($p = 0.005$), but had no significant or consistent effect in the first rep. The fungicide by invasion interaction was not significant in either replication.

Discussion

Numerous studies have reported on the potential for garlic mustard to use “novel weapons” to affect soil biota and neighboring plants (Stinson et al. 2006, Callaway et al. 2008, Anderson et al. 2010, Barto et al. 2010a, Barto et al. 2011), however relatively few native perennial herbs have been tested, and the overall impacts on the forest understory community are unclear. In this study, I tested the effects of garlic mustard-invaded soils

on seeds and seedlings of 13 perennial herbs native to North American oak woodlands and hypothesized that garlic mustard would have a negative effect on seed germination, seedling establishment and mycorrhizal colonization of roots. I found that while garlic mustard did reduce seed germination and mycorrhizal colonization of native herbs, the magnitude of these impacts was relatively small, and the effects on seedling establishment appear to vary by species and depend on environmental conditions.

Effects on Seed Germination

Garlic mustard-invaded soils negatively affected germination rates of native perennial herbs. This finding is consistent with the results of several previous studies examining the effects of garlic mustard extracts on germination (Roberts and Anderson 2001, Prati and Bossdorf 2004, Barto et al. 2010b, Pisula and Meiners 2010). Allelopathic inhibition of germination has been reported in other members of the Brassicaceae family as well (Bialy et al. 1990, Brown and Morra 1996, Kiemnec and McInnis 2002, Vaughn et al. 2006, Bainard et al. 2009). However, not all studies have found garlic mustard to have negative effects on seed germination; results appear to differ by both methods employed and the species tested. In a bioassay of four crop species, McCarthy and Hanson (1998) concluded there was little evidence of allelopathic effects by garlic mustard on seed germination, despite using concentrations of extracts that exceeded field levels. Studies applying different methods and extracts or chemical fractions led to different conclusions even for the same test species (McCarthy and Hanson 1998, Pisula and Meiners 2010). In a greenhouse study using activated carbon and garlic mustard-cultivated soils, Prati and Bossdorf (2004) found that garlic mustard's inhibitory effect on germination depended both on the origin of garlic mustard (native versus invaded range) and the species of *Geum* tested; native to North America, *G. laciniatum* experienced allelopathic responses to garlic mustard regardless of origin, whereas the European native, *G. urbana*, only responded negatively to garlic mustard from Europe. The present study also suggests that native species differ in their sensitivity to garlic mustard's inhibitory effects. Although garlic mustard significantly reduced germination across all species,

two of the eight species had higher (though non-significant) germination in garlic mustard-affected soils. Recruitment of native herbs may decline overall as a result of garlic mustard invasion, but not all species will be similarly affected.

Field studies of garlic mustard's impact on recruitment in natural settings are lacking, although there is evidence of auto-allelopathy of garlic mustard seeds (Baskin and Baskin 1992, McCarthy 1997), and reported increases in abundance of tree seedlings following garlic mustard removal (McCarthy 1997, Stinson et al. 2007) which suggest the potential for garlic mustard to affect germination in invaded forests. The present study of garlic mustard's effects on native seed germination may provide more field-relevant results than previous greenhouse studies, as it is one of the first to investigate germination in field-collected soils without supplementation of garlic mustard extracts. Even at a 25% field soil inoculum rate, germination was impacted by former occupancy by garlic mustard. The trade-off of using un-modified field soils, however, is the inability to distinguish between potential mechanisms of impact. Direct allelopathy (Prati and Bossdorf 2004, Barto et al. 2010b) and indirect AMF-mediated effects (Callaway et al. 2008) have both been implicated as pathways by which garlic mustard may reduce seed germination, and either pathway could be responsible for the observed reductions in seed germination in the current study.

The apparent effects of garlic mustard on timing of seed germination, though not significant, warrant further study. Germination timing can affect plant fitness, competitiveness and survival (Ross and Harper 1972, Miller 1987). To my knowledge, there are no published reports of garlic mustard's potential impacts on germination timing, although delayed germination has been observed in response to other Brassicacea species (Brown and Morra 1996, Kiemnec and McInnis 2002, Haramoto and Gallandt 2005). In the present study, however, there was a trend toward accelerated germination across all species. One possible explanation for these findings may be that increased nutrients in garlic mustard-invaded soils stimulated early germination. Soil chemistry is known to affect seed germination (Baskin and Baskin 2001, Fenner and Thompson

2005); in particular, the stimulatory effects of nitrogenous compounds have been documented, although species appear to differ in their response to nitrogen application and the interactions between nitrogen and other environmental variables can be complex (Steinbauer and Grigsby 1957, Popay and Roberts 1970, Williams 1983, Perez-Fernandez et al. 2006, Ochoa-Hueso and Manrique 2010, Sirová et al. 2011). Although field soils were cut with sand and potting soil to minimize nutrient differences, sufficient differences in soil chemistry may have remained and contributed to differences in germination timing. If that is the case, these differences may be even more pronounced in undiluted soils.

Effects on Seedling Establishment

The antifungal properties of garlic mustard extracts are well-documented (Roberts and Anderson 2001, Callaway et al. 2008, Cantor et al. 2011), therefore I expected garlic mustard's effects on seedling establishment and fungal root colonization to mimic the effects of fungicide. Specifically, I predicted that both garlic mustard and fungicide application would reduce AMF colonization and seedling biomass, as garlic mustard has been shown to reduce tree seedling growth via its toxic effects on AMF (Stinson et al. 2006), and benomyl is one of the most effective fungicides for suppressing AMF (Schreiner and Bethlenfalvay 1997, Smith et al. 1999, Smith et al. 2000, Callaway et al. 2004). However, I found that while garlic mustard and fungicide had similar effects on AMF colonization, their effects on seedling growth were quite different. Both invaded soils and fungicide application generally decreased AMF colonization, although the reductions were not as substantial as reported in some studies. In some field studies, benomyl has reduced AMF colonization by 80% or more (Smith et al. 2000, Callaway et al. 2004), but in this experiment, fungicide-induced reductions averaged only 14% in Rep. 1 and 6% in Rep. 2. Benomyl suppresses growth and reproduction of fungi but does not necessarily kill them (Bollen and Fuchs 1970); initiating applications at the time of seeding may have yielded more observable results than delaying application until after cotyledon stage. It is also possible that the shorter duration of this study minimized

observable effects, or that an increased application rate may have achieved greater results (Jordan and Huerd 2008). Garlic mustard's effect on AMF was somewhat stronger than that of the fungicide, with average reductions of 23% in Rep. 1 and 9% in Rep. 2 (although in Rep. 2, there was a significant invasion by species interaction; invasion reduced AMF by 25% in *Symphyotrichum cordifolium* but increased AMF by 6% in *Solidago flexicaulis*).

The modest reductions in AMF colonization caused by garlic mustard and fungicide did not necessarily lead to reduced seedling biomass. The effect of garlic mustard invasion on biomass varied by experimental replication; it produced the expected negative effect in Rep. 1, but had a positive effect in Rep. 2. Fungicide, on the other hand, had a positive effect on seedling biomass in both experimental replications. In other words: seedlings appeared to benefit from garlic mustard-invaded soils and fungicide application in the second experimental replication, but in Rep. 1, seedling biomass responded differently to the two treatments, showing a negative response to garlic mustard invasion. Biomass allocation also differed between invasion and fungicide treatments; root fraction was higher in invaded soils, but lower in fungicide-treated soils compared to non-invaded and untreated soils. These unexpected results raise several questions: Why did garlic mustard invasion and fungicide have different effects on seedling biomass? Why did seedlings respond positively to treatments that reduced AMF? And why did seedling biomass respond differently to garlic mustard invasion in the two experimental replications?

The different responses of seedling biomass to invasion and fungicide treatments suggest different pathways of impact; for example, the two treatments may be affecting different species of AMF, and/or affecting seedlings through non-target effects, such as non-AM fungi or other soil biota. If AMF species differ in their sensitivity to garlic mustard extracts and fungicide, the composition of the AMF community may shift, subsequently affecting plant growth responses. Although AMF species typical of pot experiments have been characterized as generalists, recent evidence suggests that

species-specificity in the plant-AMF relationship may be more common than previously thought (McGonigle and Fitter 1990, Dhillion 1992, Bever et al. 1996, Vandenkoornhuyse et al. 2003, Smith and Read 2008), such that plant species may respond positively or negatively to changes in AMF composition (Bever 2002, Klironomos 2003). Recent studies have had mixed results on garlic mustard's potential effects on AMF composition. In an examination of three woodland herb species collected from within and outside of naturally occurring garlic mustard populations, Burke (2008) found no significant differences in AMF root colonization rates, but did find significantly different AMF species composition within the roots of *Maianthemum racemosum*, suggesting that garlic mustard may selectively suppress AMF. However, Koch et al. (2011) observed different results in a greenhouse bioassay: garlic mustard extracts affected AMF growth but had no effect on AMF richness or composition. Similarly, while many studies using benomyl to reduce AMF report effects on overall abundance (e.g. colonization rates), different fungal species, and even different AMF species, have been shown to vary considerably in their response to benomyl application (Bollen and Fuchs 1970, Schreiner and Bethlenfalvay 1997). Although most examinations of garlic mustard's AMF-mediated impacts, present study included, have focused on overall amounts of AMF, a more nuanced approach that considers impacts on and through AMF composition may prove to be informative.

Garlic mustard invasion and fungicide may also affect seedling biomass through non-AMF pathways. Different treatment effects on non-AM fungi or other soil organisms may explain why even non-mycorrhizal seedlings responded differently to invasion and fungicide. For example, *Carex brevior* had significant positive biomass responses to fungicide treatments, but no response to garlic mustard invasion; non-AM fungal colonization rates in this species responded to both invasion and fungicide treatments, although not consistently across experimental replications. Non-AMF pathways of impact are also a reasonable hypothesis for why many mycorrhizal species responded positively to the experimental treatments, despite overall reductions in AMF. If the benefits of reduced plant pathogens outweighed the negative impacts of reduced AMF, the net effect

may be positive plant growth. Although benomyl is often used in AMF studies because of its minimal non-target effects on plants and non-AM fungi (Paul et al. 1989, Smith et al. 2000, Callaway et al. 2004, Jordan and Huerd 2008), in some cases benomyl has been found to positively affect plants by reducing pathogenic fungi and root-infecting nematodes (Rodriguez-Kabana and Curl 1980, Koide et al. 1988, Van der Putten et al. 1990, Carey et al. 1992). Although I did not assess nematode infection or characterize the pathogenicity of fungi in this study, I did quantify root colonization by non-AM fungi to determine if this might explain the positive response of seedling biomass to fungicide application. Fungicide did suppress colonization by non-AM fungi in the second experimental replication, but it had no significant effects on colonization in Rep. 1, so suppression of non-AM fungi does not appear to provide a full explanation for the observed positive responses of seedling biomass to fungicide.

Surprisingly, colonization of roots by non-AM fungi was higher in invaded soils than in non-invaded soils. The effect of invaded soils on non-AM fungi was significant in Rep. 1, which is also when garlic mustard had a negative effect on seedling biomass. In Rep. 2, when garlic mustard had a positive effect on seedling biomass, overall rates of non-AM fungal colonization were substantially lower than in Rep. 1, and garlic mustard had no significant effect on non-AM fungi. I am not aware of any published reports of garlic mustard's impacts on non-AM fungi and other soil organisms, with the exception of unpublished data reported at a symposium by Blossey et al. (2005) that indicated that accumulation of soil pathogens may contribute to declines in garlic mustard vigor over multiple generations; they further suggested that such pathogen accumulation might also negatively impact native plant communities, although this effect was not demonstrated. Byproducts of glucosinolates (e.g. cyanide compounds) found in garlic mustard and other Brassicacea species have been found to be toxic to some plant pathogens; in fact, mustard crops have been used to "biomfumigate" crop fields because of these anti-pathogen properties (Kirkegaard et al. 1996, Kirkegaard and Sarwar 1998). However, the results of this study suggest that garlic mustard may enhance populations of some non-AM fungal species, and that this may be another pathway through which garlic mustard can impact

native plants. Higher rates of non-AM fungal colonization may be an example of pathogen accumulation, in which exotic plants accumulate pathogens in the rhizosphere but are less sensitive to them than co-occurring native species (Eppinga et al. 2006, Inderjit and van der Putten 2010), or the result of pathogen protection by AMF (Newsham et al. 1995, Pozo and Azcon-Aguilar 2007). The fact that AMF colonization rates were significantly lower, and non-AM fungal colonization was significantly higher in invaded soils (Rep. 1) supports the latter hypothesis. The potential for complex pathways of impact, in which garlic mustard may affect seedlings through both AMF and non-AM fungal communities cannot be confirmed by this study but certainly merits further examination.

My original hypothesis that garlic mustard would negatively affect native seedlings by reducing AMF rested on the assumption that AMF would provide a benefit to native seedlings, as has been observed in other woodland herb species (Lapointe and Molard 1997, Whigham 2004). However, another potential explanation for the positive response of seedlings to AMF-reducing treatments, in addition to the non-AMF pathways described above, is that AMF are not, in fact, providing a benefit to these seedlings. Plants sustain a carbon cost to maintain the mycorrhizal association. In situations where the carbon cost outweighs the benefits received, the net effect of the mycorrhizal association may be negative, resulting in reduced plant growth (Walling and Zabinski 2006, Shah et al. 2009). This shift in the plant-AMF relationship from symbiosis to parasitism has been observed in high-nutrient environments (Johnson et al. 1997, Rowe et al. 2007). When nutrient levels are high, plants may not require AMF for nutrient acquisition, and the relationship is no longer beneficial to the plant. Although in this experiment, field soils were cut with sand and no fertilizer was applied in order to maintain nutrient-limited conditions, phosphorus levels were very high in the field soils used, and it is possible that plants in this study were not sufficiently nutrient-depleted within the course of this study to require the AMF relationship, hence the positive response to AMF-reducing treatments.

The unexpected differences in seedling biomass response to garlic mustard invasion in the two experimental replications also support the hypothesis that the AMF-plant relationships shifted from symbiosis to parasitism. Although nutrient conditions were the same in each replication, climatic conditions affecting soil moisture and plant stress were not. As previously described, a malfunction in the climate control system resulted in very high peak temperatures in Rep. 1, with a maximum temperature exceeding 40 °C on multiple days (Figure 2). The excessive heat caused desiccation in many seedlings, and signs of stress (e.g. purpling leaves) were observed. By the second experimental replication, the greenhouse climate controls were addressed, temperatures were considerably milder and less variable, and at no point in Rep. 2 did plants experience desiccation or other obvious environmental stress. These environmental differences may explain why AMF colonization appeared to benefit seedlings in Rep. 1, but not in Rep. 2. Under the stressful environmental conditions of Rep. 1, seedlings were more susceptible to drought, and possibly also more vulnerable to plant pathogens, hence the higher rates of non-AM fungi observed in Rep. 1 than in Rep. 2. AMF may therefore have benefitted seedlings both by improving their drought tolerance (Allen and Boosalis 1983, George et al. 1992, Augé 2001), and by reducing their vulnerability to pathogens (Newsham et al. 1995, Pozo and Azcon-Aguilar 2007). In Rep. 2, by comparison, seedlings experienced minimal environmental stress and may therefore have been less-vulnerable to pathogens and drought; in these conditions AMF may have exacted a carbon cost without providing substantial benefit in return. Within-species patterns of biomass and AMF also appear to support this hypothesis: In Rep. 1, *Solidago flexicaulis* and *Symphyotrichum cordifolium* had both had lower AMF colonization and biomass in invaded soils, suggesting negative impacts of AMF reductions, while in the invasion treatment in Rep. 2, *S. cordifolium* had lower AMF but higher biomass, and *S. flexicaulis* had higher AMF, but lower biomass, again suggesting that AMF may not have been providing a benefit to seedlings in Rep. 2, but instead may have been acting parasitically.

One additional explanation for the different responses to invaded soils between experimental replications is that soil attributes—either biotic or chemical—changed

while in storage between the two experiments. Lankau (2010) found that garlic mustard's allelopathic inhibition of sycamore (*Platanus occidentalis*) seedlings depended on the presence of soil biota; no allelopathic responses were observed in sterilized soil, and study results suggested that some soil microbes may degrade the allelochemicals, rendering them less potent. It is therefore possible that the negative biomass responses to invaded soils in Rep. 1 were caused by direct allelopathy, but that the responsible allelochemicals had been degraded by soil microbes prior to the start of Rep. 2. However, the fact that negative responses to invaded soils were observed in the seed germination study—which occurred simultaneously with Rep. 2 of this seedling establishment study—suggests that allelopathic or harmful soil biota were still present during this time.

Mechanisms of Impact and Management Implications

Garlic mustard may impact herb population dynamics in invaded woodlands by reducing native seed germination, AMF colonization of roots, and potentially increasing vulnerability to non-AM fungi, either directly via pathogen accumulation, or indirectly via the reduction of AMF. The effects of garlic mustard invasion on seedling establishment, however, may depend on species and environmental conditions. Contrary to expectations, garlic mustard does not appear to mimic the effects of the fungicide benomyl, which is often used in experimental investigations of AMF impacts. Although both garlic mustard and benomyl have anti-fungal properties, their pathway of impact on seedling growth may differ in ways that can affect experimental outcomes. Future research on garlic mustard's AMF-mediated effects should include effects on and through AMF species composition and diversity, as well as interactions with non-AM fungal species and other soil biota.

The interactions of biotic and abiotic soil mechanisms are also an important avenue of future research, as demonstrated by studies showing interactive effects of garlic mustard allelopathy with AMF and other soil biota (Barto et al. 2010b, Lankau 2010) and competitive effects (Cipollini et al. 2008b). In this study, potential allelopathy cannot be

clearly distinguished from biotic soil pathways. However, Barto and Cipollini (2009b) found that garlic mustard metabolites have very short half-lives, ranging from 3-12 hours in non-sterile soil. Field concentrations of garlic mustard extracts are often too low to be detectable, although concentrations likely vary seasonally with garlic mustard life cycles (Haribal and Renwick 2001, Barto and Cipollini 2009b, Cantor et al. 2011). Cantor et al. (2011) found that garlic mustard's secondary metabolites (AITC and sinigrin) were most frequently detected in July and August, coinciding with second-year plant senescence. The field soils for this study were collected approximately two months following adult senescence, and therefore allelopathic extract levels were likely quite low at the time of collection, suggesting that biotic pathways were more likely responsible for the results observed in this study. This assumption is further supported by a greenhouse study using activated carbon that found no evidence of direct allelopathic impacts of garlic mustard on similar oak woodland herbs, including *Solidago flexicaulis* (Van Riper et al. 2008). Regardless, the altered biotic—and potentially abiotic—soil conditions caused by garlic mustard invasion persisted in the greenhouse environment in the absence of garlic mustard plants, suggesting the possibility of a soil legacy effect that may continue to affect native seedlings even after successful management of garlic mustard (Corbin and D'Antonio 2012).

Although small in magnitude, the negative effects of garlic mustard on native herb seed germination are concerning, both from a conservation and restoration standpoint. Native woodland plants are already declining due to numerous anthropogenic and ecological stressors, and reduced germination rates may threaten the long-term viability of some plant populations. Although vegetative reproduction is more common in many woodland herbs (Bierzychudek 1982, Jolls 2003), reproduction by seed enables plant populations to adapt to a changing environment (Jump and Peñuelas 2005). By reducing seed germination, garlic mustard may further compromise opportunities for native plant populations to adapt to the current and future changing climate conditions. Reduced germination success may also limit opportunities for woodland restoration. In many systems, particularly grasslands, restoration by seed is a more cost-effective method than

planting plugs, but restoration of woodland herbs by seed is challenging, due to specific germination requirements, lengthy dormancy, slow seedling growth, and high mortality (Bierzychudek 1982, Cullina 2000, Mabry 2005, Mottl et al. 2006, Drayton and Primack 2012). This study suggests that woodland restoration may face additional challenges when attempting to seed into invaded or formerly-invaded areas. Fortunately, the reductions in germination observed in this study were, while significant, fairly moderate for most species. Woodland herb restoration by seed may therefore still be a viable option in invaded areas, with increased seeding rates to compensate for garlic mustard's effects. Further investigation into garlic mustard's effects on germination, including potential effects on germination timing, will be important for more fully understanding the potential long-term impacts on plant populations, as well as the potential to restore native woodland herbs.

Garlic mustard's impacts on seedling establishment are less straightforward, as they may depend on local environmental conditions. In nutrient-rich soils typical of many North American woodlands, AMF may not be an important pathway of impact. Garlic mustard may be reducing AMF, but with negligible impacts on native herb seedling growth. In more nutrient-limited sites, or conditions of stress, i.e. drought or herbivory, the AMF pathway may be more important. Hahn and Dornbush (2012) observed interactive effects of garlic mustard and herbivory by exotic slugs; garlic mustard negatively affected survival of juvenile herbs only when combined with a slug herbivory treatment. They hypothesized that slug herbivory might weaken herbs, leaving them more susceptible to garlic mustard competition. It may be instead that under the stress of herbivory, herbs experienced impacts of reduced AMF that were not apparent in unstressed herbs.

Because garlic mustard has also been found to increase nutrient availability in woodlands (Rodgers et al. 2008b), the AMF pathway may also be more important early in the invasion process, and become less important over time. This departure from the AMF mechanism may be further exacerbated by observed declines in the potency of

phytochemicals and increased resistance among AMF communities following initial invasion (Lankau et al. 2009, Lankau 2011). In this study, native seedlings had a positive response to garlic mustard-invaded soils when environmental conditions were ideal. It does not necessarily follow, however, that such benefits will be realized in natural field settings. Soil-mediated impacts are not the only way that garlic mustard interacts with native plants—competition is also believed to be an important mechanism of impact (Meekins and McCarthy 1999, Cipollini and Enright 2009). The same conditions that may render AMF less beneficial to native plants—nutrient-rich soils and abundant moisture—are also known to favor garlic mustard (Byers and Quinn 1998, Meekins and McCarthy 2001, Hewins and Hyatt 2010). Native seedlings may therefore be impacted by garlic mustard even in resource-rich sites, but primarily through the competition pathway as opposed to the soil biota pathway. Studies that aim to test the relative effects of multiple pathways of impact under a range of environmental conditions should yield a more complete understanding of community level responses to garlic mustard invasion and management.

Overall, this study of garlic mustard’s impacts on 13 native perennial herbs suggests that garlic mustard negatively affects germination and seedling establishment under certain conditions. However, it is important to note that individual species in this study did not respond uniformly to garlic mustard-invaded soils. Significant soil invasion by species interactions were observed in multiple response variables, including AMF and non-AM fungal colonization rates and seedling biomass. This suggests that responses to soil biota and garlic mustard invasion are not likely to be uniform, and that we can expect some “winners and losers” among native plant species in invaded woodlands (McKinney and Lockwood 1999, Wiegmann and Waller 2006). For example, *Mitella diphylla* appears to be more sensitive to garlic mustard’s effects on seed germination than the other species tested; with significantly reduced germination rates and a trend toward accelerated germination timing. Although this study did not provide clear patterns of which species were most likely to increase or decline as a result of garlic mustard invasion, it does reemphasize the potential for shifts in community composition and

successional trajectories in response to invasion (Rodgers et al. 2008a), and cautions against assuming community level responses to garlic mustard based on the responses of individual species. Efforts to identify winners and losers may also be useful in identifying candidates for restoration. Species that are less sensitive to garlic mustard's below-ground impacts may be good candidates for restoration following garlic mustard control, whereas more sensitive species may require additional care or amelioration of altered soil conditions when reintroducing.

Table 3-1. Species planted in seedling germination and establishment experiments, and the seed stratification treatments, number planted, and percent germination for the germination study.

Species	Common Name	Family	Seed Stratification (days)	# Seeds per Pot	% Germination (mean)	
					Invaded	Non-invaded
<i>Allium tricoccum</i> *	wild leek	Liliaceae	warm-moist (16); cold-moist (40)	200	0	0
<i>Carex brevior</i>	plains oval sedge	Cyperaceae	cold-moist (60)	75	87.7	79.7
<i>Desmodium glutinosum</i>	pointed-leaf tick trefoil	Fabaceae	cold-moist (12)	30	24.0	32.0
<i>Festuca subverticillata</i>	nodding fescue	Poaceae	cold-moist (60)	64	76.9	86.3
<i>Geranium maculatum</i> *	wild geranium	Geranaceae	cold-moist (40)	67	3.6	4.2
<i>Hydrophyllum virginianum</i>	virginia waterleaf	Hydrophyllaceae	warm-moist (16); cold-moist (40)	82	0	0
<i>Mitella diphylla</i>	bishop's cap	Saxifragaceae	cold-moist (60)	210	3.5	11.2
<i>Osmorhiza claytonii</i>	sweet cicely	Apiaceae	warm-moist (16); cold-moist (40)	98	0	0
<i>Phlox divaricata</i> ⁺	wild blue phlox	Polemoniaceae	cold-moist (60)			
<i>Scrophularia marilandica</i>	late figwort	Scrophulariaceae		900 ^x	30.4	31.9
<i>Solidago flexicaulis</i>	zig-zag goldenrod	Asteraceae	cold-moist (60)	43	50.7	46.5
<i>Symphotrichum cordifolium</i>	heart-leaved aster	Asteraceae	cold-moist (60)	456 ^x	21.7	27.9
<i>Thalictrum dioicum</i>	early meadow-rue	Ranunculaceae	warm-moist (16); cold-moist (40)	84	0	0

* Species used in germination study only.

⁺ Species used in establishment study only.

^x Seed number estimated based on seed weight.

Table 3-2. Comparison of invaded and non-invaded soils collected from Warner Nature Center (Marine-on-St. Croix, MN) for use in greenhouse experiments. Soil analysis was conducted by University of Minnesota Soil Testing Laboratory (St. Paul, MN).

	Invaded	Non-invaded
Soil texture	Medium (loam, silt loam)	Medium (loam, silt loam)
Organic matter (%)	4.9	3.4
pH	5.7	5.2
Nitrate (ppm)	7.5	5.3
Phosphorus (ppm)	49	50
Potassium (ppm)	138	89
Calcium (ppm)	1339	696
Magnesium (ppm)	144	107

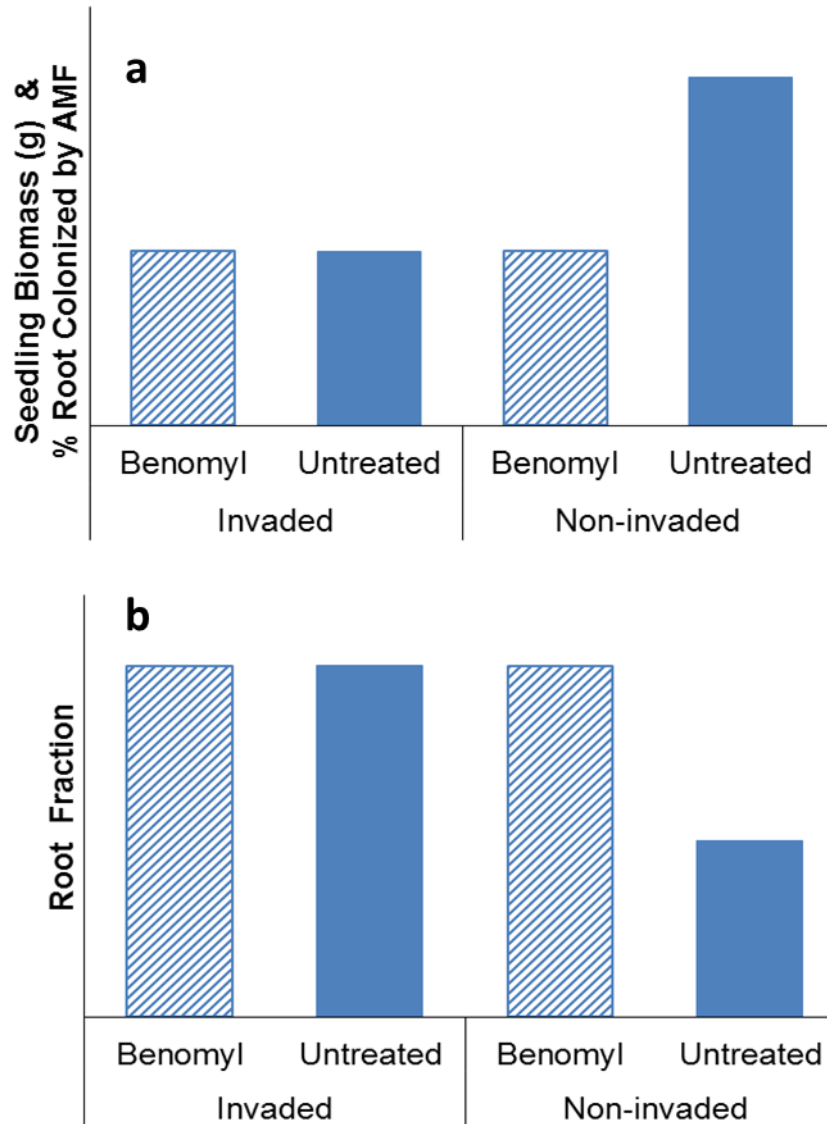


Figure 3-1. Predicted responses of seedling biomass and colonization of roots by AMF (**a**) and root fraction (**b**) to garlic mustard invasion and fungicide (benomyl) application. Root fraction = root biomass/total biomass. I predicted that the effects of garlic mustard invasion would be similar to the effects of fungicide application in that both treatments would inhibit AMF colonization and therefore disadvantage native seedlings, resulting in lower total biomass and greater allocation of biomass to roots. I also predicted a

fungicide by invasion treatment interaction, in which the effects of benomyl application would be more pronounced in the non-invaded soils, due to the expected higher levels of AMF present in non-invaded soils.

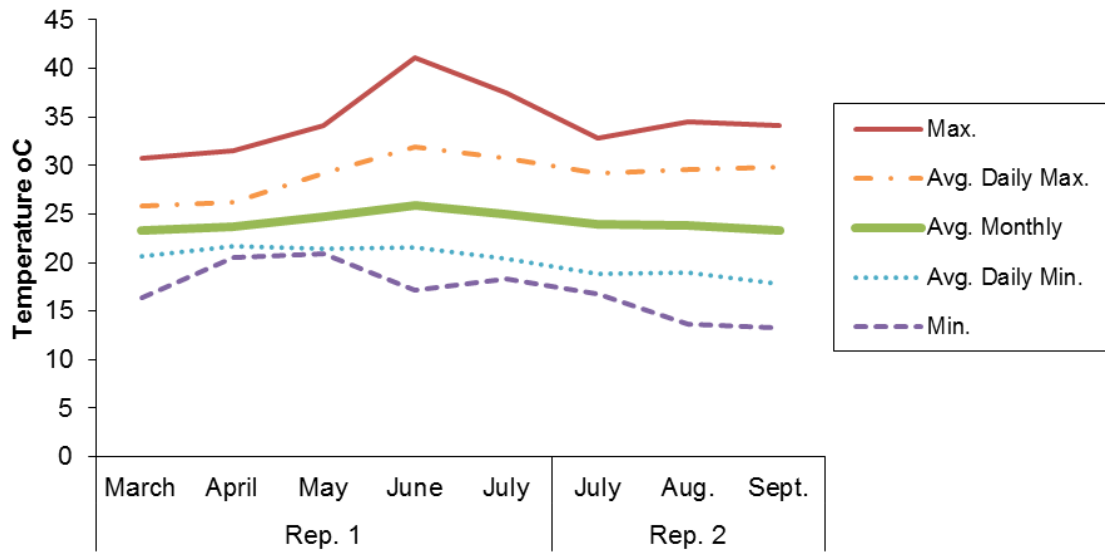


Figure 3-2. Greenhouse temperature mean and range during two replications of the seedling establishment experiment: Rep. 1: March 10 – July 8; Rep. 2: July 12 – Sept. 30. The germination experiment took place during the Rep. 2 time period. Maximum and minimum temperatures represent the extreme temperatures reached in a given month, while average daily maximum and minimum represent the mean high and low daily temperatures averaged over the month.

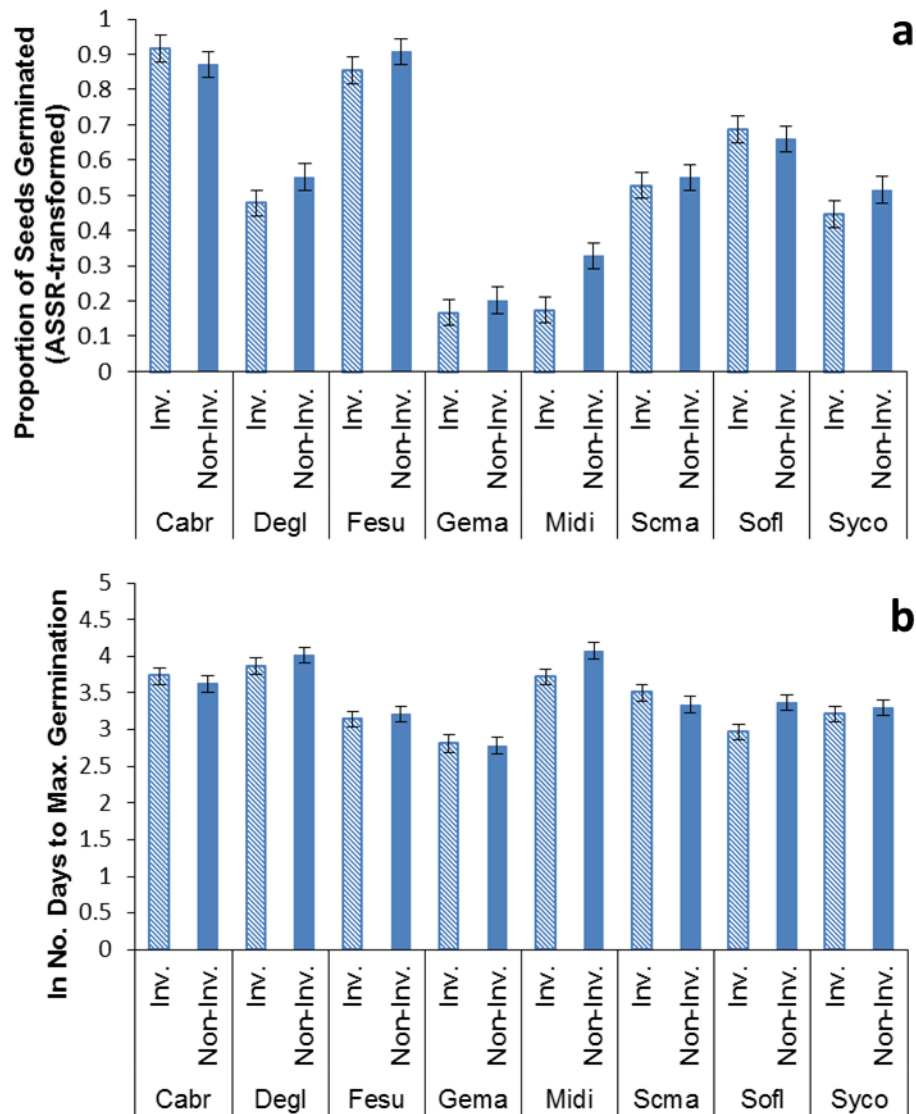


Figure 3-3. Seed germination percentage **(a)** and timing **(b)** of eight native species in soils from garlic mustard-invaded and non-invaded areas. Germination differed significantly by species ($p < 0.001$) and by invasion history ($p = 0.03$), with overall lower germination in soils from garlic mustard-invaded areas. The species by invasion interaction was not significant. Days to reach peak germination differed significantly by species and was slightly lower in invaded soil than non-invaded soil ($p = 0.09$). The species by invasion interaction was not significant. Bars represent least squares means \pm 1 SE. Cabr = *Carex brevior*; Degl = *Desmodium glutinosum*; Fesu = *Festuca*

subverticillata; Gema = *Geranium maculatum*; Midi = *Mitella diphylla*; Scma = *Scrophularia marilandica*; Sofl = *Solidago flexicaulis*; Syco = *Symphyotrichum cordifolium*.

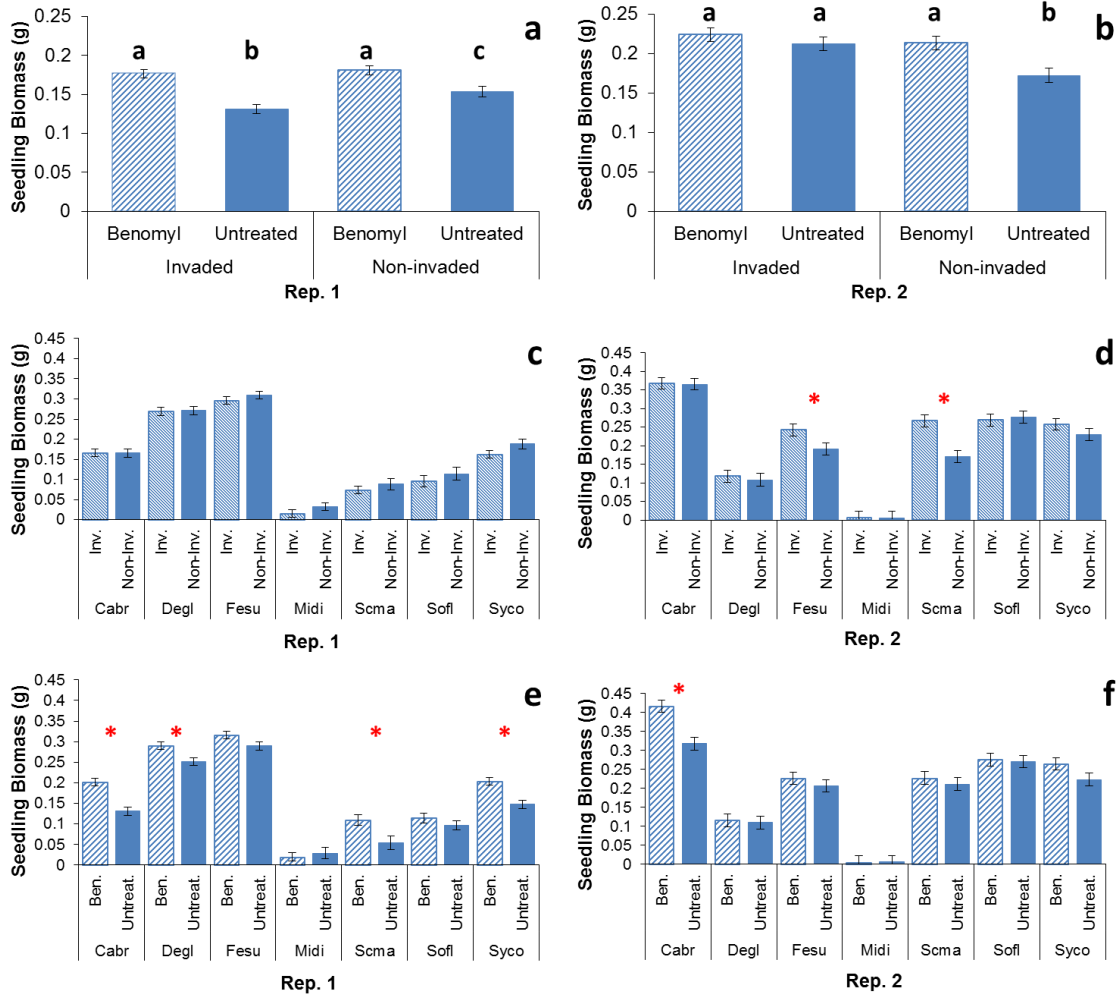


Figure 3-4. Observed patterns of seedling biomass in response to garlic mustard-invaded soils and fungicide (benomyl) application in two greenhouse experiment replications. Overall treatment effects: **a)** in Rep. 1, seedling biomass was significantly lower in invaded soils ($p = 0.03$), and fungicide had a positive effect on seedling biomass within both invaded ($p < 0.0001$) and non-invaded ($p = 0.003$) soils. **b)** In Rep. 2, biomass was significantly higher in the fungicide treatment in non-invaded soils only ($p = 0.001$). Although in both replications, the effects of invasion were most pronounced within untreated soils, the invasion by fungicide interaction was not significant in either replication. Within the untreated soils, biomass was significantly lower in invaded soils in Rep. 1 ($p = 0.008$), and significantly higher in invaded soils in Rep. 2 ($p = 0.002$). Effects of garlic mustard-invaded soils: **c)** Seedling biomass was significantly lower in

the invasion treatment in Rep. 1 ($p = 0.03$); the within-species treatment effect was nearly significant in *S. cordifolium* ($p = 0.07$). **d**) In Rep. 2, there was a significant invasion by species interaction ($p = 0.02$). Seedling biomass response to fungicide treatment in **e**) Rep. 1 and **f**) Rep. 2: fungicide application had a positive effect on seedling biomass in most species, and the species by fungicide treatment interactions were significant in both replications ($p = 0.006$ and $p = 0.04$, respectively). Within species, treatment effects were also nearly significant in *F. subverticillata* in Rep. 1 ($p = 0.06$), and in *S. cordifolium* in Rep. 2 ($p = 0.07$). Shown are least squares means ± 1 SE. Letters indicate statistically significant differences between treatments ($p < 0.05$). Asterisks indicate significant within-species differences by treatment ($p < 0.05$). Cabr = *Carex brevior*; Degl = *Desmodium glutinosum*; Fesu = *Festuca subverticillata*; Midi = *Mitella diphylla*; Scma = *Scrophularia marilandica*; Sofl = *Solidago flexicaulis*; Syco = *Symphyotrichum cordifolium*.

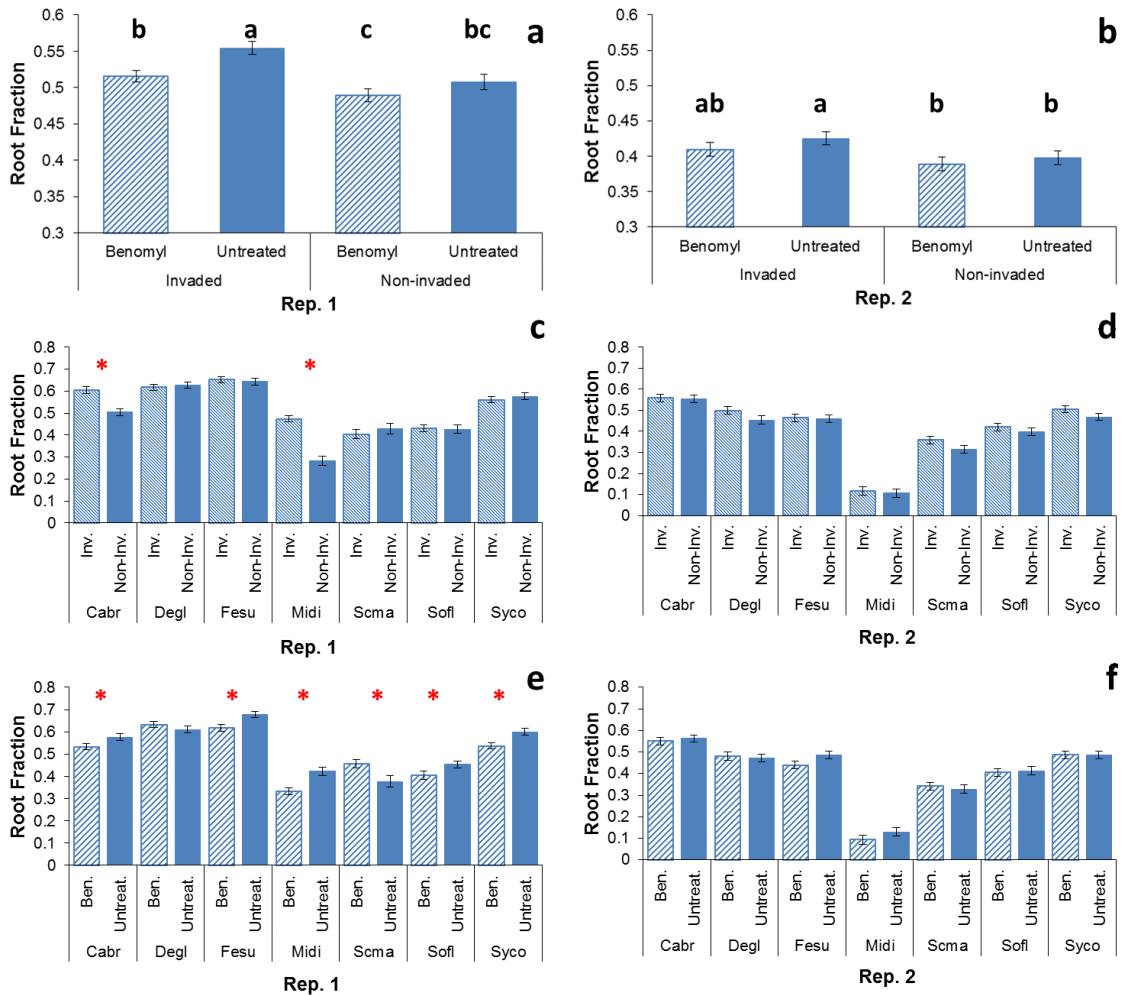


Figure 3-5. Observed patterns of root fraction as a response to garlic mustard-invaded soils and fungicide (benomyl) application. Overall, root fraction was higher in invaded soils and lower in benomyl-treated soils. **a)** In Rep. 1, both the invasion by species and fungicide by species interactions were significant ($p < 0.0001$ and $p = 0.0001$, respectively); **b)** in Rep 2, only the invasion and species main effects were significant ($p < 0.0001$ and $p = 0.01$, respectively). The invasion by fungicide interaction was not significant in either replication of the greenhouse experiment. Response to garlic mustard invaded soils in two replications of a greenhouse experiment: **c)** There was a significant invasion by species interaction in Rep. 1 ($p < 0.0001$); **d)** in Rep. 2, root fraction was consistently higher in invaded soils; invasion and species main effects were significant ($p = 0.02$ and $p < 0.0001$, respectively). In Rep. 2, treatment effects within *D.*

glutinosum and *S. marilandica* were nearly significant ($p = 0.08$). Response to fungicide application: **e**) The fungicide by species treatment interaction was significant in Rep. 1 ($p = 0.0001$); **f**) neither fungicide main effects nor treatment interactions were significant in Rep. 2. There were no significant within-species treatment effects in Rep. 2, although the effect of fungicide was nearly significant in *F. subverticillata* ($p = 0.06$). Shown are least squares means ± 1 SE. Letters indicate statistically significant differences between treatments ($p < 0.05$). Asterisks indicate significant within-species differences by treatment ($p < 0.05$). Cabr = *Carex brevior*; Degl = *Desmodium glutinosum*; Fesu = *Festuca subverticillata*; Midi = *Mitella diphylla*; Scma = *Scrophularia marilandica*; Sofl = *Solidago flexicaulis*; Syco = *Symphotrichum cordifolium*.

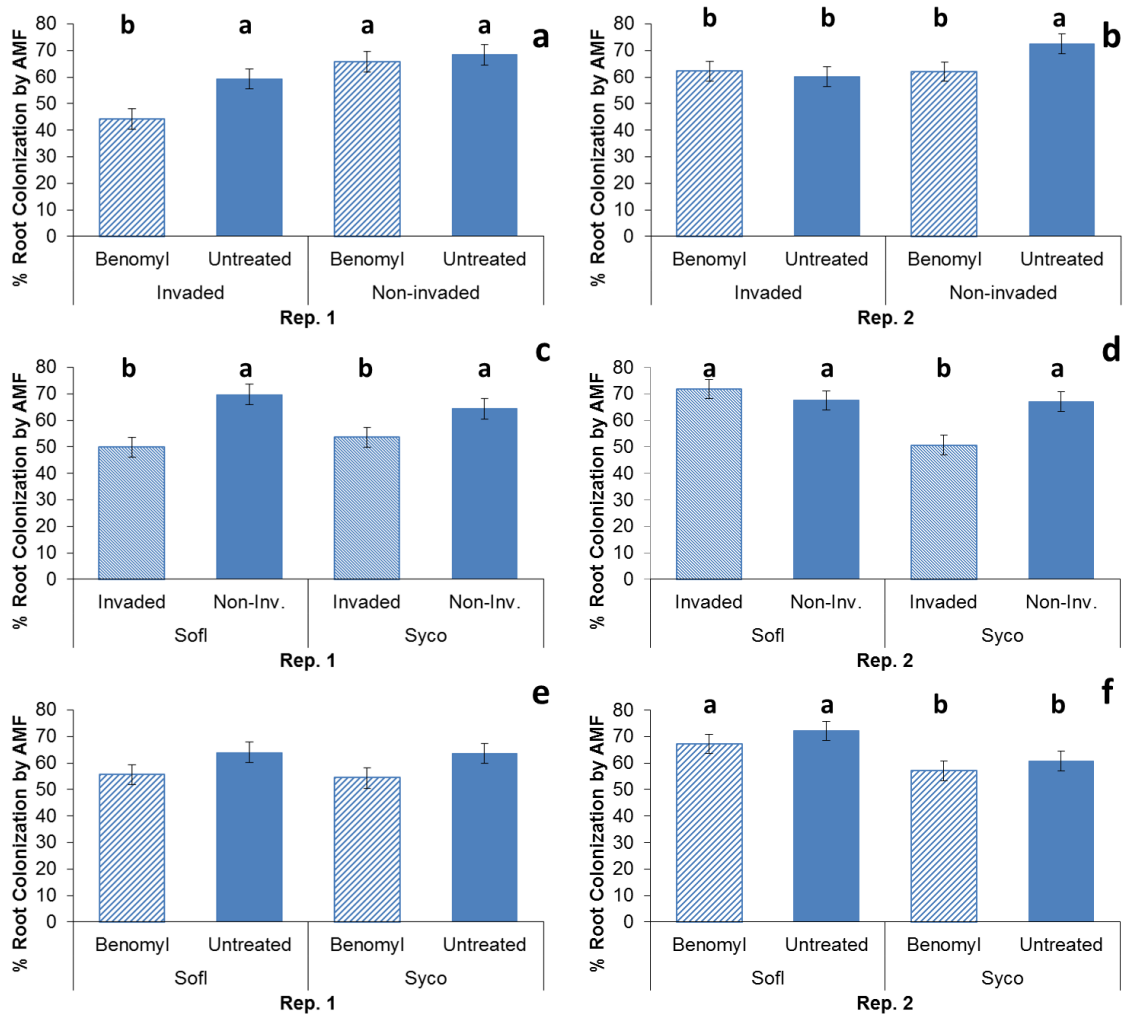


Figure 3-6. Effects of garlic mustard invaded-soils and fungicide (benomyl) application on AMF colonization of two native herbs in two replications of a greenhouse experiment. Shown are overall treatment effects in **a**) Rep. 1 and **b**) Rep. 2; within-species effects of invaded soils in **c**) Rep. 1 and **d**) Rep. 2; and within-species effects of fungicide treatment in **e**) Rep. 1 and **f**) Rep. 2. In Rep. 1, AMF colonization rates were significantly lower in invaded and benomyl-treated soils ($p = 0.0001$ and 0.02 , respectively). In Rep. 2 there was a significant invasion by species interaction ($p = 0.006$). Although the effects of benomyl appear stronger in invaded soils in Rep. 1, and in non-invaded soils in Rep. 2, the fungicide by invasion interaction was not significant in either replication (although nearly so in Rep. 2; $p = 0.09$). Shown are least squares means \pm 1 SE. Letters indicate

statistically significant differences ($p < 0.05$). Sofl = *Solidago flexicaulis*; Syco = *Symphyotrichum cordifolium*.

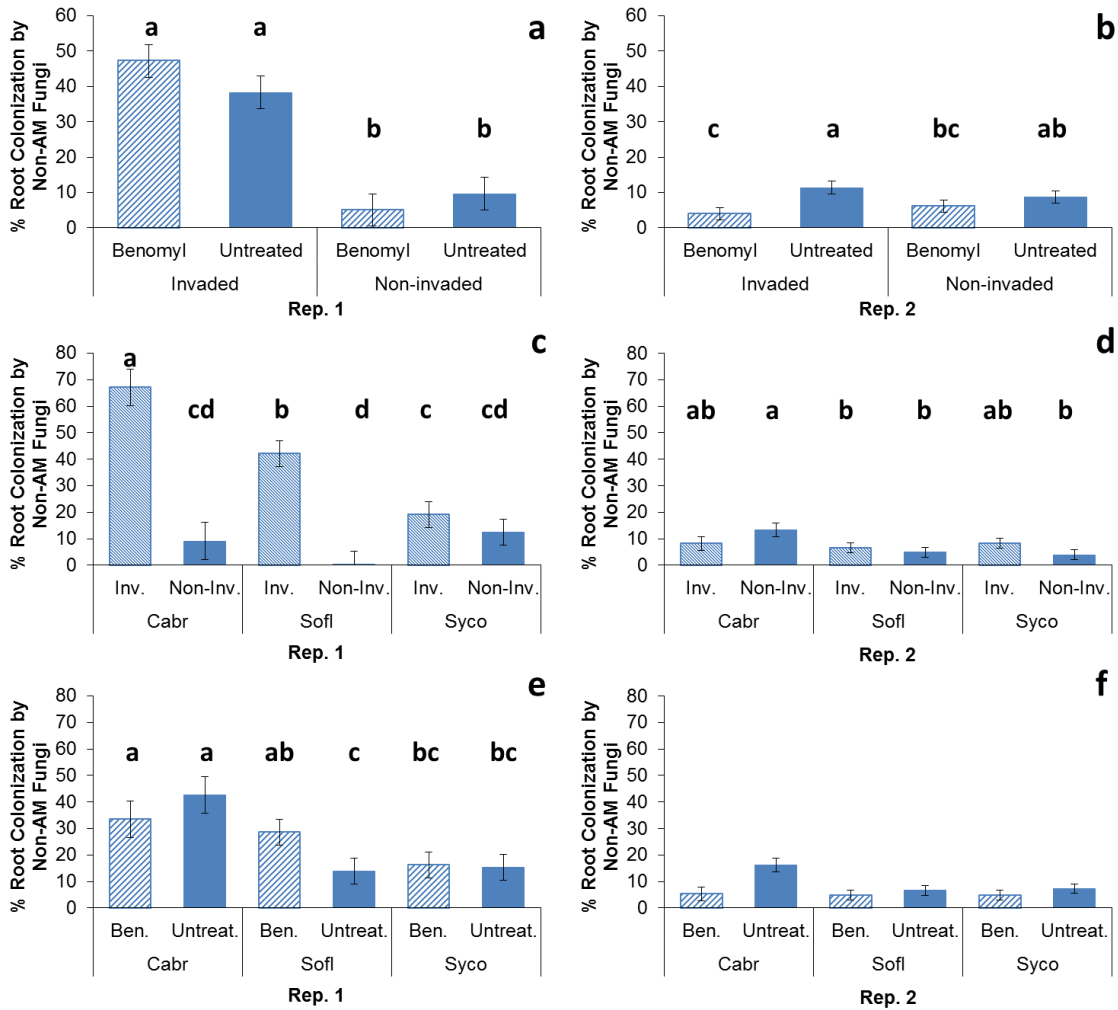


Figure 3-7. Effects of garlic mustard invaded-soils and fungicide (benomyl) application on non-AM fungal colonization of three native species in two replications of a greenhouse experiment. Shown are overall treatment effects in **a)** Rep. 1 and **b)** Rep. 2; within-species effects of invaded soils in **c)** Rep. 1 and **d)** Rep. 2; and within-species effects of fungicide treatment in **e)** Rep. 1 and **f)** Rep. 2. In Rep. 1 there was a significant invasion by species interaction ($p < 0.0001$), but the fungicide main effect and interactions were not significant. In Rep. 2, fungicide significantly reduced colonization rates ($p = 0.005$); the species main effect was nearly significant ($p = 0.06$), but invasion and all interactions were not. Shown are least squares means \pm 1 SE. Letters indicate statistically significant differences ($p < 0.05$). Cabr = *Carex brevior*; Sofl = *Solidago flexicaulis*; Syco = *Symphyotrichum cordifolium*.

Chapter 4

Effects of Native Plant Cover, Species Richness and Light Availability on Garlic Mustard (*Alliaria petiolata*) Invasion

The degree to which invasive species drive or respond to environmental change has important implications for management and restoration. The invasive herb garlic mustard (*Alliaria petiolata*) is often implicated as a driver of change in North American woodlands, yet its interactions with native herbs are poorly understood. Inverse relationships between garlic mustard and native plants may result from garlic mustard's impacts, but some native plants have been found to be competitive with garlic mustard, and observations suggest that garlic mustard invasion may respond to differences in native plant cover and resource availability. In this study, I tested the effects of native herb richness, cover and light on the invasibility of garlic mustard in a dry-mesic oak woodland in East Central Minnesota. I planted 50 garlic mustard seeds into blocks of experimental plots that were previously planted with native herbs in a range from 0 to 10 species. I measured garlic mustard seedling establishment, survival to rosette and adult stages, and average (per plant) and total (per plot) adult biomass and silique production. Using structural equation models, I analyzed the direct, indirect and net effects of light, native richness and cover on successive garlic mustard life stages.

Native plant cover had a significant negative effect on all garlic mustard life stages. Species richness had no direct effect on garlic mustard but had a significant positive effect on native cover, resulting in indirect negative effects on all garlic mustard stages, and net negative effects on adult numbers, total biomass, and average and total silique production. Light had a negative direct effect on garlic mustard seedling establishment and a positive effect on native plant cover, resulting in significant negative indirect and net effects of light on garlic mustard seedling, rosette and adult numbers. However, via apparent density dependence in garlic mustard, the net effect of light on total biomass and

silique production was positive. The interacting effects of plant cover, richness and light suggest that woodlands lacking a diverse and robust native groundcover may be more vulnerable to garlic mustard invasion. High light levels may indirectly accelerate invasion, as the lack of inter- and intra-specific competition increases garlic mustard's reproductive output. Garlic mustard invasion may thus be, in part, a response to native plant decline. Restoring woodland herb communities and addressing other drivers of environmental change may reduce garlic mustard invasion.

Introduction

The Eurasian herb garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] is considered a threat to North American hardwood forests because of its rapid rate of spread (Nuzzo 1999, Scott 2000, Meekins and McCarthy 2002, Evans and Landis 2007), ability to alter soil chemistry (Rodgers et al. 2008b) and biota (Vaughn and Berhow 1999, Roberts and Anderson 2001, Cipollini 2002, Stinson et al. 2006, Burke 2008, Callaway et al. 2008, Wolfe et al. 2008, Anderson et al. 2010, Barto et al. 2011), and potential impacts on native plant communities (McCarthy 1997, Meekins and McCarthy 1999, Nuzzo 2000). Negative relationships between garlic mustard and native species diversity and abundance observed both in time (Nuzzo 1991) and space (Van Riper et al. 2010) are often cited as evidence that garlic mustard may harm native plant species. However, there are other potential explanations for these patterns: garlic mustard may be responding to declines in native species, or garlic mustard and native species may both be responding in opposite directions to environmental change. These three explanations are not mutually exclusive.

Often implicated as a driver of change in North American woodlands (Rodgers et al. 2008a), garlic mustard's potential competitiveness (Anderson et al. 1996, Meekins and McCarthy 1999) and impacts on native ecosystems has been the focus of much of the research on garlic mustard, as well as the justification for ongoing management and biocontrol research (Nuzzo 1991, Blossey et al. 2001a). However, while garlic mustard

has been found to suppress some native species via competition (Meekins and McCarthy 1999, Cipollini and Enright 2009) and chemically-induced impacts on soil biota (Stinson et al. 2006), studies testing whether these impacts are responsible for declining native diversity or abundance have yielded conflicting results (McCarthy 1997, Hochstedler et al. 2007, Stinson et al. 2007, Rooney and Rogers 2011). Declines in the abundance and diversity of native woodland herbs have been observed in many North American woodlands (Brewer 1980, Robinson et al. 1994, Rooney et al. 2004, Rooney and Rogers 2011) as a result of direct losses from over-harvesting (Jolls 2003); environmental impacts of human activities such as logging (Duffy and Meier 1992, Meier et al. 1995, Small and McCarthy 2002), agriculture (Singleton et al. 2001, Flinn and Vellend 2005), and urbanization (Drayton and Primack 1996); increased herbivory pressure from white-tailed deer (*Odocoileus virginianus* Zimmerman (Rooney and Waller 2003, Côté et al. 2004, Webster et al. 2005, Wiegmann and Waller 2006) and exotic slugs (Hahn et al. 2011); as well as the impacts of invasive plants (Woods 1993, Gould and Gorchoff 2000, Collier et al. 2002, Frappier et al. 2003, Greene and Blossey 2011) and earthworms (Bohlen et al. 2004, Frelich et al. 2006, Nuzzo et al. 2009). However, the possibility that garlic mustard may be responding to, rather than (or in addition to) driving these declines in native plant species, has received little attention.

Although the effect of native plant communities on garlic mustard invasion has not yet been formally tested, there is evidence to suggest that garlic mustard may respond to declines in native plants, and that native plant cover and diversity may contribute to invasion resistance. Garlic mustard is often observed flourishing in disturbed sites that lack native ground cover (Trimbur 1973, Nuzzo 1991, Van Riper et al. 2010). As native plants decline, the resulting environmental changes may create more-suitable conditions for garlic mustard. Most notably, increased light levels, moisture, and nutrient availability (Anderson et al. 1996, Hewins and Hyatt 2010), and decreased litter levels (Trimbur 1973, Bartuszevige et al. 2007) may all contribute to garlic mustard invasion. Declines in both overall abundance and diversity of native species can increase local

resource availability, contributing to invasion of exotic plants (Davis et al. 2000, Naeem et al. 2000, Fargione and Tilman 2005).

Furthermore, some native plant species appear to compete effectively against garlic mustard and may potentially inhibit garlic mustard invasion (Meekins and McCarthy 1999, Murphy 2005, Bauer et al. 2010). Analyses of native plant response to garlic mustard removal and herbicide treatments has not typically shown strong evidence of competitive release (Carlson and Gorchoff 2004, Hochstedler et al. 2007, Bauer et al. 2010), particularly in the case of perennial herbs (McCarthy 1997, Stinson et al. 2007). This suggests either that native herb recovery is inhibited by other (non-garlic mustard) factors; or that response to removal is lagged due to dispersal limitation (Brudvig et al. 2011) or soil legacy effects (Corbin and D'Antonio 2012); or that some native plants were competitive against garlic mustard and therefore not suppressed by garlic mustard invasion. In a greenhouse experiment, Meekins and McCarthy (1999) explicitly tested the competitive interactions between garlic mustard rosettes and three native species and found that when growing at high densities, the herbaceous annual *Impatiens capensis* and seedlings of the tree *Acer negundo* were equally or more competitive than garlic mustard, respectively. Competition for light was suggested as a likely mechanism, because both of these species overtopped garlic mustard rosettes in the experiment, however the authors questioned whether sufficiently high densities of *A. negundo* would occur in a natural woodland setting. In a field experiment, Murphy (2005) found that planting even moderate densities of the perennial herb *Sanguinaria canadensis* L. (bloodroot) was capable of suppressing garlic mustard at multiple life stages.

Like many plant species, garlic mustard also exhibits intraspecific competition: garlic mustard plants growing in high densities have lower survival, biomass and reproductive output, on average, than do individuals growing in lower densities (Trimbur 1973, Meekins and McCarthy 2002, Rebek and O'Neil 2006). Also, second-year garlic mustard plants have been shown to competitively suppress garlic mustard seedlings (Baskin and Baskin 1992, Winterer et al. 2005, Pardini et al. 2009, Herold et al. 2011),

potentially through shading (Bauer et al. 2010), suggesting that garlic mustard seedlings in particular may also be vulnerable to competitive pressures of other vegetation (i.e. interspecific competition). We might expect to see lower seedling survival, plant biomass, and reproductive output when garlic mustard grows in dense native vegetation. Bauer et al. (2010) suggested that first-year garlic mustard plants may be vulnerable both to competition from second-year plants prior to native plant emergence in early spring, as well as to native vegetation later in the growing season. The loss of native plant cover may thus release garlic mustard from competitive effects and facilitate invasion.

Species richness may further contribute to the competitive effects of native plants on garlic mustard and thus reduce invasion. On a local scale, more-diverse plant communities utilize available resources more completely, resulting in greater overall productivity (Tilman et al. 1996, Tilman et al. 2001, Hooper et al. 2005), and reducing the availability of resources for potential invaders (Naeem et al. 2000, Kennedy 2002, Fargione and Tilman 2005). A species-rich forest understory may therefore be less vulnerable to invasion by garlic mustard due to higher cover and lower resource availability.

In this experiment, I hypothesized that a species-rich woodland herbaceous community would effectively compete with invading garlic mustard for light, which is often a limiting resource of the forest understory (Tremblay and Larocque 2001, Neufeld et al. 2003, Whigham 2004), influencing overall understory productivity (Ellison and Houston 1958), as well as garlic mustard growth and reproduction (Dhillion and Anderson 1999, Meekins and McCarthy 2000, 2001, Myers et al. 2005, Eschtruth and Battles 2009b). Light levels and disturbances that create patches of higher light availability (e.g. roadsides, timber clearings, and invasive tree removal) are often found to facilitate invasion by garlic mustard and other exotic species in forests (Parendes and Jones 2000, Webb et al. 2001). If native herbs can effectively compete with and inhibit garlic mustard across a range of light levels, then woodlands with a diverse and dense

understory community may be more effective at suppressing garlic mustard invasion following canopy-opening disturbances.

I tested the interacting effects of light availability, native plant richness and cover on the invasibility of garlic mustard by planting garlic mustard seeds across a range of native species richness and cover and measuring garlic mustard establishment, survival, growth, and silique (seed capsule) production. Garlic mustard seeds were planted into plots of established native herbs (located adjacent to an existing garlic mustard population), offering a rare opportunity to experimentally explore the initial stages of invasion in a field setting. I hypothesized that if native plant cover negatively impacts garlic mustard performance, then garlic mustard would have lower germination rates, survival, biomass and silique production when planted into plots with higher cover. Species richness could have both direct negative effects on garlic mustard performance, as well as indirect negative effects mediated through increased plant cover. If garlic mustard benefits from lower native richness or cover, it would suggest that garlic mustard invasion may be responding to declines in native species in woodland systems. I further hypothesized that light would have direct positive effects on both native cover and garlic mustard responses, resulting in an indirect negative effect on garlic mustard as mediated through native cover. The net effect of light on garlic mustard would depend on the relative strength of the positive and negative effects of light and cover respectively.

This study is unique in that, to my knowledge, it is the first to explicitly and experimentally test the effects of diversity on garlic mustard invasion. It also expands on previous studies of the competitive interactions between garlic mustard and native plants by examining the effects of multiple species (i.e. a simulated community) on garlic mustard responses, as opposed to the two-species interactions tested by Meekins and McCarthy (1999) and Murphy (2005). Additionally, the design allows me to test both the direct and indirect effects of richness, cover and light on successive life stages of garlic mustard, providing a richer explanation of the complex interacting drivers of invasion.

Understanding the combined effects of light, native cover and richness on garlic mustard will help elucidate the mechanisms of garlic mustard invasion and invasion resistance in woodlands. Garlic mustard is generally thought to be a superior competitor, but the potential ability of diverse native herb communities to reduce invasion by decreasing light availability to garlic mustard has not been investigated. In addition to theoretical implications, this study has important practical implications for management, such as the ability to identify woodlands most vulnerable to invasion, as well as informing strategies for reducing invasibility. If garlic mustard is causing declines in native species, then the logical management priority is to prevent and control garlic mustard invasions. However, if garlic mustard is responding to declines in native species, then it may be reasonable to focus management efforts on restoring native plants and addressing the primary causes of native decline. Furthermore, if native plant diversity or cover decreases invasibility, than restoration of the native plant community may play an important role in reducing further invasion.

Methods

Study Site

This study was conducted in a dry-mesic oak forest at Warner Nature Center in Marine-on-St. Croix, Minnesota, 35 km northeast of the city of Saint Paul. The forest canopy is dominated by oak (*Quercus alba* L., *Q. rubra* L.), maple (*Acer rubrum* L., *A. negundo* L.), and cherry (*Prunus serotina* Ehrh.), and the most common understory species include *Rubus* spp. L., *Athyrium filix-femina* (L.) Roth, *Rhamnus cathartica* L. (seedlings), *Circaea lutetiana* L., *Galium aparine* L., *Geum canadense* Jacq. and *Desmodium glutinosum* (Muhl. ex Willd.) Alph. Wood (L. Van Riper, unpublished data). Other ground-layer species frequent in the immediate study area include *Amphicarpaea bracteata* (L.) Fernald, *Thalictrum thalictroides* (L.) Eames & B. Boivin, *Eurybia macrophylla* (L.) Cass., *Geranium maculatum* L., *Parthenocissus* sp. Planch., *Maianthemum racemosum* (L.) Link ssp. *racemosum*, *Stellaria media* (L.) Vill., and

Trientalis borealis Raf. (Taxonomy follows USDA, NRCS (2011)). Like many urban and suburban forests, the site has abundant populations of white-tailed deer (*Odocoileus virginianus*) and is heavily invaded by buckthorn (*Rhamnus cathartica*), garlic mustard, European earthworms and slugs. Standing litter levels are low, and bare ground is common (Van Riper et al. (2010) and personal observation). The soil in the immediate study area is Kingsley sandy loam (Soil Survey Staff 2010) with 68% sand, 15% clay, and 17% silt (Knight 2006).

Field Plots

The study was conducted in field plots that were originally established in 2003 for a buckthorn invasion study (see Knight (2006) for details). Fifteen 2-by-3 meter blocks were placed across a range of light levels (canopy openness ranging from 1% to 15% as measured with an LAI-2000 plant canopy analyzer). Within each block, four 0.5-by-0.5 meter plots (60 plots total) were cleared of existing vegetation and planted at four levels of native species richness: 0, 3, 6, and 10 species of common woodland herbs (Table 1). The positions of the plots were randomly selected within each block, and the species composition of each 3- and 6-species plot was randomly selected from the pool of 10 species. The planted species were allowed to establish, grow and reproduce from 2003 to 2005. After the first year, species that experienced mortality were not replaced. Unplanted colonizing species were removed annually from 2003 through 2006, but not prior to final data collection in 2007. In 2005, the present study was designed in response to observations of a rapidly-expanding garlic mustard population adjacent to the plots.

Garlic Mustard “Invasion” and Data Collection

In November 2005, I planted 50 garlic mustard seeds into each plot to simulate the initial stages of invasion following seed-set of a single garlic mustard plant (Meekins and McCarthy 2001). Seeds were collected from adult plants at the study site the previous August, cleaned to remove silique pods and chaff, and stored in paper envelopes at 2° C.

I prepared the plots for planting by removing leaf litter and roughing the soil surface with a hand cultivator. I then scattered the seeds evenly over the plot, patted them into the soil surface, and replaced the litter layer over the seeds.

In May 2006, I counted the number of garlic mustard seedlings that established in each plot. The following November, I again counted the garlic mustard plants in each plot to determine survival to the rosette stage. In July 2007, I counted the number of adult plants, quantified silique production (average per plant and total per plot), and harvested the aboveground biomass, which was then dried for 7 days at 60°C, weighed, and used to calculate per plant averages and plot totals. Biomass was not harvested from one block, so analysis of the total and average biomass included data from 14 blocks (56 plots).

Treatments and Environmental Variables

Pre-invasion native species richness and cover were measured in 2005 (Knight 2006). Total percent cover per plot was estimated as the total area of the plot that would be occupied by leaf area of all individuals rooted in the plot, such that cover estimates were not constrained to 100%. In 2007, using the same methodology, I quantified the number of individuals of each native species to determine the current species richness and estimated the total percent cover of native plants in each plot. Individuals that colonized the plots in 2006 and 2007 were included in the calculations of species richness and cover, with the exception of newly-germinated seedlings (contributing < 1% cover). Cover estimates were not available for 2006, but cover appeared to increase linearly between 2005 and 2007 ($r^2 = 0.92$; $p < 0.0001$; proc corr; SAS 9.2), so an estimate of cover per plot for the 2006 growing season was obtained by averaging the cover estimates from 2005 and 2007.

Light is often a limiting resource in the forest understory (Tremblay and Larocque 2001, Neufeld et al. 2003, Whigham 2004), driving much of plant productivity (Ellison and Houston 1958). The amount of light that can penetrate to the herb layer is affected by the tree canopy openness. To account for light's effect on native plant cover and

garlic mustard invasion, I used data from Knight (2006), who characterized the growing season canopy openness (light levels) above each plot by averaging the diffuse non-interceptance (DIFN) light levels recorded with LAI-2000 plant canopy analyzers (Li-Cor Inc., Lincoln, NE, USA) over the months of April through September, 2004-2005. To calculate the percentage of light penetration through the overstory canopy, below-canopy measurements obtained above each plot were divided by above-canopy measurements obtained from a unit placed in a nearby open field, monitoring sky conditions every 15 seconds. Measurements were taken in overcast conditions to measure ambient light levels and minimize the influence of diurnal variation in sun angle. I was unable to repeat this methodology during the years of the study, however light data collected on a single day in July with a Li-Cor quantum sensor was correlated with the 2004-2005 canopy openness averages ($r^2 = 0.67$; $p < 0.0001$; proc corr; SAS 9.2). I used the LAI data for the analysis, because this method best explains mean daily photosynthetic photon flux density in shaded understories, and because measurements taken with the LAI-2000 correlate well to repeated measurements over time (Machado and Reich 1999).

Soil pH, nitrogen, and phosphorus were also measured in order to characterize the site and account for the distribution of the plots (grouped within blocks). To analyze soil pH, I collected soil samples from each plot (10 cm depth) in early September, 2007. The soil was air-dried and mixed with water in a 1:2 ratio. The pH of the resulting slurry was measured with a Beckman 10 pH meter (Beckman Coulter, Inc., Brea, CA, USA). To measure the availability of phosphorus (P), nitrate (NO_3^-) and ammonium (NH_4^+) in each plot, I buried a nylon bag containing 15 ml of acid-washed anion-exchange resins at approximately 10 cm depth. The bags were buried on April 22 and collected on July 30, 2007 (for a total of 99 days) to measure nutrient levels during the adult garlic mustard growing season. Resins were kept frozen until extraction. To extract the nutrients, resin bags were thawed and rinsed in nanopure water, air-dried for one week and weighed. The dried resins were placed in 30-ml syringes with a glass microfiber filter and rinsed with 100 ml of 2M NaCl in 0.1M HCl. The extract was transferred to plastic culture tubes and frozen. Soluble reactive phosphorus (SRP) was analyzed using the methods of

Strickland and Parsons (1972). Nitrogen was analyzed colorimetrically on an Alpkem RFA 300 Autoanalyzer at the Soil Testing Laboratory of the University of Minnesota, St. Paul, MN (<http://soiltest.cfans.umn.edu/>).

Statistical Analysis

I used structural equation models (SEM; Amos Graphics, Student Edition) to analyze the effects of light (canopy openness), species richness and cover on garlic mustard response variables (Figure 1). SEM is a type of multivariate regression analysis that tests hypothetical interactions, or pathways, between multiple variables (Grace 2006). The advantage of the SEM approach for analyzing ecological processes is that it allows the testing of both direct and indirect (or net) effects of multiple variables simultaneously. With SEM analysis, I was able to test not only the direct effects of light, species richness and cover on garlic mustard response variables, but also the indirect effects of light and richness as mediated through their effects on native cover. Additionally, SEM allowed me to test the effects on successive life stages of garlic mustard by separating out the initial effects on seedling establishment from effects on later life stages. The number of established garlic mustard seedlings was included as both a response and a predictor of the number of garlic mustard rosettes, adults, and adult biomass and silique production. Seedling number was hypothesized to have a positive effect on the number of rosettes and adults, as well as total (per plot) biomass and silique production. However, seedling number was expected to have negative effects on average (per plant) biomass and silique production due to density-dependent effects. Average biomass and silique production data was natural log-transformed to improve normality.

To account for the spatial grouping of plots within blocks, I used nonmetric multidimensional scaling (NMS; PC Ord 5.32) to spatially distinguish plots based on environmental variables. The primary matrix included light, pH, NO_3^- , NH_4^+ , and P, and the secondary axis included the plot identification. The axis produced by NMS ($r^2 = 0.98$) was defined primarily by NO_3^- ($r^2 = 0.94$), and to a lesser extent by P ($r^2 = 0.22$). This axis was included in the SEM as a synthetic “block effect” variable. Soil nutrients

and pH were used to define block effects, but their effects were not explicitly tested in the structural equation models. Errors of species richness and both block effect and light levels were positively correlated in the specified models (richness and block effect: 0.36 and 0.23 in years 1 and 2, respectively; richness and light: 0.13 and 0.35 in years 1 and 2, respectively).

To increase comparability of factors measured in different units, all estimates reported are standardized (in standard deviation units), such that an increase of one standard deviation in a factor results in a change in the response variable equal to the number of standard deviations specified by the estimate, while holding all other conditions constant (Grace and Bollen 2005). Standard errors and statistical significance are bootstrap estimates calculated in Amos Student Edition.

Results

Species Richness and Cover

In 2005, species richness in the plots ranged from 0 to 10 species, and the mean species richness was 3.8 species per plot (Table 2). Richness was slightly lower in 2007, ranging from 0 to 8 species per plot, with a mean of 2.9. Although species richness declined from 2005 to 2007, overall mean percent cover of native species increased from 55% to 79%. Native percent cover ranged from 0% to 187% (in 2005) and 250% (in 2007). Non-planted species—primarily vines—occurred in eight plots (13% of plots) in 2007, 4 of which were originally 0-species plots. Within these eight plots, non-planted colonizers contributed an average of 14% cover (100% of the total non-garlic mustard plant cover in 0-species plots, and 11% of the total plant cover in planted plots). Non-planted species were included in calculations of 2007 species richness and cover. The exotic species *Stellaria media* occurred in a single plot contributing less than 10% total cover. It was included in the calculations of species richness and cover despite its non-native status.

Garlic Mustard Establishment, Survival, Biomass and Silique Production

An average of 8.2 garlic mustard seedlings established per plot, or 16.4% of the original 50 seeds planted (Table 3). Establishment rates were variable, ranging from 2 – 36%. Another 1.13% (mean 0.57 ± 0.14 SE) of the seeds established in 2007, but these seedlings did not affect the results and are excluded from the analysis in this paper. On average, 75.5% of established seedlings in 2006 survived to the rosette stage, and 87.5% of rosettes survived to adulthood. In 2007, the average number of adults in each plot was 5.7, or 11.3% of the seeds planted. The number of seedlings that established had a strong positive effect on the number of rosettes at the end of the first year ($p = 0.002$; Figure 2; Tables 4 and 5), as well as the number of adult plants in the second year ($p = 0.001$; Figure 3; Tables 4 and 5). However, seedling number had a significant negative effect on average biomass ($p = 0.001$) and average silique production per plant ($p = 0.03$; Tables 4 and 5).

Effects of Native Plant Cover and Richness and Light on Garlic Mustard

Native plant cover had a significant negative effect on all stages of garlic mustard invasion, including seedling establishment ($p = 0.02$), survival to rosette stage ($p = 0.002$) and adulthood ($p = 0.002$), average and total biomass ($p < 0.001$ and $p = 0.001$, respectively), and average and total silique production ($p = 0.001$; Figures 2 and 3; Tables 4 and 5). Although species richness did not have consistent or statistically significant direct effects on garlic mustard, it did have a significant positive effect on native plant cover ($p = 0.001$), causing a significant negative indirect effect on all garlic mustard response variables, and a negative total effect (direct effect plus indirect effect mediated through cover) on the number of adult garlic mustard plants ($p = 0.004$), total biomass production per plot ($p = 0.002$), and average and total silique production ($p = 0.035$ and 0.002 , respectively).

Light had a strong negative direct effect on garlic mustard seedling establishment ($p = 0.002$) and positive but non-significant direct effects on later life stages. Light also had a

positive effect on native plant cover ($p = 0.003$ in 2006; not significant in 2007), which contributed to significant indirect and total effects of light on the number of garlic mustard seedlings ($p = 0.006$ and $p = 0.002$), rosettes ($p = 0.001$) and adults ($p = 0.001$ and $p = 0.002$). However, light had positive but non-significant direct and indirect (mediated through native cover and the number of garlic mustard seedlings) effects on all biomass and reproductive measures, resulting in significant positive total effects on total biomass ($p = 0.018$) and total silique numbers per plot ($p = 0.048$), and a trend of positive total effects on average biomass ($p = 0.069$) and average silique numbers per plot ($p = 0.063$).

Discussion

In this study, I tested the interacting effects of understory light, species richness and cover on garlic mustard invasion in woodlands. As hypothesized, native plant cover had a strong negative effect on all garlic mustard life stages. Species richness did not directly affect garlic mustard, but had negative indirect effects mediated through increased plant cover. The effects of light differed by garlic mustard life stage and were dependent on native plant cover: light mediated through cover had a net negative effect on the numbers of garlic mustard plants, but a net positive effect on total biomass and silique production per plot. A diverse and robust woodland understory may therefore suppress but not prevent garlic mustard invasion and spread.

Effects of Plant Cover and Species Richness on Garlic Mustard Invasion

As predicted, native plant cover had a significant negative effect on garlic mustard establishment, survival, biomass, and reproduction (Figures 2 and 3; Table 4), suggesting that garlic mustard is sensitive to competitive pressures from surrounding vegetation. Bauer et al. (2010) hypothesized that garlic mustard seedlings would be sensitive to competition for light with native plants, as has been suggested for seedlings of invasive species in other ecosystems, but this study confirms Murphy's (2005) findings that later life stages are sensitive to competitive effects as well. Thus, a robust herbaceous ground

cover may play an even stronger than expected role in resisting garlic mustard invasion, as it not only reduces the initial number of seedlings that establish, but also reduces overall survival, productivity and reproduction of garlic mustard plants. In particular, the negative effects of cover on both average silique production per plant, and total silique production per area, suggests that a dense native groundcover may slow not only initial rates of invasion, but rates of spread as well.

Species richness contributed significantly to native cover in both years of the study, and therefore had a significant indirect negative effect on all garlic mustard responses (Figures 2 and 3; Table 4). Plots with higher species richness had higher percentages of native cover, possibly due to spatial niche partitioning. The species in the study plots comprised different growth forms, including both low-growing groundlayer species (e.g. *Galium boreale*, *Hydrophyllum virginianum*, and *Viola pubescens*), and taller species with a more upright growth habit (e.g. *Maianthemum racemosum*, *Solidago flexicaulis*, and *Thalictrum dioicum*). Thus, woodland plant communities with higher local species richness might be capable of supporting higher overall cover than those containing fewer species, particularly if those species have similar growth habits.

The effect of species richness on cover may have been partly due to the particular species planted in this experiment. Included were species that contributed greatly to cover through rapid spread (e.g. *Solidago flexicaulis* and *Hydrophyllum virginianum*), as well as a species that, protected from deer herbivory, attained a large, dense growth habit (*Thalictrum dioicum*). Higher diversity plots would be more likely to include one of these high-cover species, and thus the effect of species richness on cover could be due to a sampling effect. However, even if diversity impacts are due to sampling effects, diversity may still be beneficial, particularly when it is unknown which species are contributing the desired effect (Hector et al. 2001).

Species richness did not, however, have a significant direct effect on garlic mustard at any life stage (Figures 2 and 3; Table 4). Local diversity is thought to reduce a site's vulnerability to invasion by more effective and complete use of resources, or by

increasing the probability that a site will contain a species that is competitive with the invader (Fargione and Tilman 2005). The lack of direct effects indicates that the primary pathway of influence on garlic mustard is through species richness' contribution to greater overall cover, as previously described. The resulting increased native plant cover may exert competitive pressures on garlic mustard through higher resource utilization, or greater appropriation of light and space. Despite the lack of consistent or significant direct effects, species richness had significantly negative total effects, mediated through cover, on the number of second-year garlic mustard plants, total biomass per plot, and both average and total silique production (Figures 2 and 3; Table 4). In a study of the effects of diversity on invasion in a California tussock ecosystem, Levine (2000) found that the effects of diversity arose at the seedling stage, presumably because seedlings are more vulnerable to shading by surrounding vegetation. However, in this experiment, effects of diversity as mediated through cover were stronger in second-year garlic mustard plants. Using the structural equation models to test both direct and indirect effects demonstrates that species richness does appear to inhibit garlic mustard invasion; this effect may not be detected via univariate statistical approaches to testing diversity-invasion relationships.

Effect of Light on Native Vegetation and Garlic Mustard

Light (canopy openness) was included in the SEM because it is often a limiting resource in the forest understory, strongly impacting herb growth and reproduction (Ellison and Houston 1958, Tremblay and Larocque 2001, Neufeld et al. 2003, Whigham 2004). Although individual species differ in their level of shade-tolerance and response to light levels (Brewer 1980, Small and McCarthy 2002, Whigham 2004), light is generally considered to have a positive effect on plant productivity at the forest floor (Ellison and Houston 1958). Therefore, I hypothesized that light would have a positive effect on native plant cover in my study plots (Fig. 1). Light has also been found to have positive effects on garlic mustard invasion (Dhillion and Anderson 1999, Meekins and McCarthy 2000, 2001, Myers et al. 2005, Eschtruth and Battles 2009b); I hypothesized that light

would have a positive direct effect on garlic mustard establishment, survival, biomass and reproduction. However, I also expected that light would have an indirect negative effect on garlic mustard, as mediated through increased native cover. The net effect of light would therefore depend on the relative strength of light's positive effect on both garlic mustard responses and native cover, and the negative effect of cover on garlic mustard invasion.

As predicted, light had a positive effect on native plant cover, although the effect was significant only in the first year of the study (Figures 2 and 3). Many herb species have been found to have higher biomass and reproduction with increased light levels (Pitelka et al. 1980, Valverde and Silvertown 1995, Routhier and Lapointe 2002). The species included in this study were summer-flowering herbs (with the exception of *Allium tricoccum*, a spring ephemeral), which have been found to be particularly sensitive to the effects of light, with higher mortality in deep shade (Brewer 1980). Through this positive effect on native plant cover, light had significant indirect negative effects on garlic mustard establishment and survival to rosette and adults stages (Table 4), as was hypothesized.

However, the direct effects of light on garlic mustard did not support my hypothesis. Although light generally appeared to have positive effects on garlic mustard survival, biomass and reproduction, these effects were not significant. Furthermore, light had a significant *negative* effect on garlic mustard seedling establishment (Figure 2; Table 4). Although this is consistent with greenhouse experiments that found higher percentages of germination in the dark versus light (Baskin and Baskin 1992), other field experiments have reported variable results regarding the effect of light on germination. Some studies have reported higher germination in woodland edges versus interiors (Meekins and McCarthy 2001), or in litter removal treatments versus controls (Bartuszevige et al. 2007), which suggest that light has a positive effect on germination, whereas other field studies have found lower germination or seedling cover associated with higher light (Byers and Quinn 1998, Van Riper et al. 2010). This inconsistency is likely due to the

interactions of light with soil moisture (Byers and Quinn 1998, Meekins and McCarthy 2001), as well as differential effects of light and/or moisture on germination and seedling survival (i.e. establishment). For example, Bartuszevige et al. (2007) found that litter removal treatments that increased light availability but decreased soil moisture had significant positive effects on garlic mustard germination but reduced seedling survival. However, in their study, the positive effect on germination was stronger than the negative effect on survival, such that there was net positive effect of litter removal on the total number of seedlings established. In this study, I measured seedling establishment but not germination. In Minnesota, garlic mustard germinates in late March (pers. obs.), and in this study, seedlings were counted in May, after their first true leaves had extended and they were easily identifiable. Garlic mustard is known to have high rates of both seed germination and seedling mortality (Trimbur 1973, Anderson et al. 1996), and this potential early mortality was not captured in this study. I therefore cannot determine to what extent light (or associated soil moisture) was affecting germination versus seedling mortality. Nonetheless, the effect of light on seedling establishment (those that germinated and survived early mortality) was clearly negative, which contradicts the findings of Bartuszevige et al. (2007).

The total effect of light on garlic mustard was a function both of light's direct effects, and the indirect effects as mediated through cover and the number of garlic mustard seedlings (Figures 2 and 3; Table 4). I predicted that the total effect would be simply the difference between its positive direct effects on garlic mustard responses and the indirect negative effects as mediated by cover. However, because light had a significant negative effect on seedling establishment, interpreting lights' total effects is somewhat more complicated, as it also reflects the influence of garlic mustard seedling number on later life stages. The number of garlic mustard seedlings was, not surprisingly, a strong positive predictor of the number of rosettes and adults, but it had significant negative effects on average biomass and average silique production per plant (and negative but non-significant effects on total biomass and silique production per plot). When garlic mustard established in higher numbers, the plants tended to be smaller and have less

reproductive output, consistent with other reports of density-dependence in garlic mustard (Trimbur 1973, Meekins and McCarthy 2002, Rebek and O'Neil 2006). Thus, the total effects of light on later garlic mustard life stages reflected this density dependence. Light had significant negative total effects on the number of rosettes and second-year plants, resulting primarily from negative indirect effects mediated through seedling number. However, the net effect of light on total (per plot) biomass and reproduction was significantly positive, reflecting positive (non-significant) direct effects of light on biomass and silique numbers, as well as light's negative effect on seedling numbers and subsequent indirect positive effect on productivity and reproduction. Light's total effects on garlic mustard biomass and silique numbers would presumably be more strongly positive, were it not also mediated through native plant cover.

Other Potential Pathways of Impact: Soil Resources

Light clearly plays an important role in the dynamics of understory plant communities, including interactions between native and invasive species. However, the results of the SEM analyses suggest that other resources, specifically soil moisture and nutrients, also likely influence garlic mustard invasion. As mentioned above, the strong negative effects of light on garlic mustard seed germination may be caused by seed or seedling desiccation. Garlic mustard seed germination has been found to be sensitive to drought (Baskin and Baskin 1992), and higher light plots might have lower soil moisture levels due to increased exposure and evaporation. Although soil moisture was not analyzed for this experiment, previous measurements in the study plots (Knight 2006), other research at this study site (Chapter 2) as well as in other woodlands (Minckler and Woerheide 1965) have actually found a positive correlation between canopy openness and soil moisture, presumably because areas with an open canopy have lower density of tree roots competing for available soil moisture. However, such areas may still be considerably drier and hotter at the immediate soil surface, creating unfavorable conditions for vulnerable seedlings. Shade from native herb cover does not appear to

counteract this effect; native cover may instead exacerbate the effect through increased competition for soil moisture and other soil resources.

Another finding that points toward the importance of competition for soil resources is that the negative effects of herb cover on garlic mustard remain strong even in the second year, when many garlic mustard plants achieve sufficient height to overtop the surrounding native vegetation. In addition to competition for soil moisture, increased native cover may limit availability of soil nutrients or physical space to garlic mustard plants. In grassland experiments, plots with higher species richness have been found to have higher overall nitrogen uptake and biomass production (Tilman et al. 1996), increased “crowding” (Kennedy 2002) and greater invasion resistance (Naeem et al. 2000, Fargione and Tilman 2005). In woodlands, species richness and cover may likewise contribute to greater utilization of soil resources, in addition to the more complete appropriation of light that penetrates the tree canopy. Although light is generally found to have a positive effect on garlic mustard growth and reproduction, garlic mustard has also been shown to have a high degree of plasticity in its response to light levels (Dhillon and Anderson 1999), as well as a phenological niche separation from native herbs that allows it to make use of pre-canopy closure light availability (Jean Engelhardt and Anderson 2011). It may be that within an acceptable range of light levels, soil resources are more critical to determining garlic mustard’s productivity and reproduction. Garlic mustard is considered a “nitrophile”, in that its productivity increases significantly in response to nitrogen enhancement, but it also has flexible nitrogen uptake strategies (Hewins and Hyatt 2010) and appears to increase availability of soil nutrients over time (Rodgers et al. 2008b). It is possible, though, that nutrients are limiting to garlic mustard’s performance early in the invasion process, and the ability to increase nutrient availability allows garlic mustard populations to overcome this limitation over time.

Regardless of which resources have the greatest influence on garlic mustard invasion, it appears that the pathway by which species richness reduces this resource(s) is through

the increase in native plant cover. In other words, it appears that richness affects resource availability through increases on overall plant volume (and, likely, demand for resources), as opposed to more efficient utilization of soil resources due to richness at a given cover level, per se. As previously described, if species richness affected garlic mustard through additional (non-cover) pathways, we would expect to see significant *direct* effects of richness on garlic mustard in the SEMs, and that was not the case. However, it is possible that the range of species richness included in this study (0 – 10 species) was not sufficient to produce a direct effect on garlic mustard, or that soil resource availability was high enough that it did not limit garlic mustard despite the effects of species richness. In other words, species richness may result in lower resource availability through complete utilization of soil resources, as has been observed in grasslands (Tilman et al. 1996), but the reduced availability has little effect on garlic mustard. Although this study does suggest that some factor other than light levels influences garlic mustard invasion, additional research is needed to determine what that factor is, and what the role of species richness and cover might be in affecting its availability and thereby indirectly affecting garlic mustard.

Implications for Garlic Mustard Invasion and Management

The results of this study support previous indications that garlic mustard is sensitive to interspecific competition from native vegetation (Meekins and McCarthy 1999, Murphy 2005, Bauer et al. 2010), as native plant cover had significant negative effects on garlic mustard establishment, survival, productivity and reproduction in first- and second-year garlic mustard plants. Additionally, the SEM models indicate that species richness indirectly suppresses garlic mustard invasion via significant contributions to understory plant cover. Together, these findings suggest that woodlands lacking a diverse and robust native groundcover may be more vulnerable to garlic mustard invasion, and that inverse relationships between garlic mustard and native plants may be caused, at least in part, by garlic mustard's response to native plant decline. Restoring native woodland herbs may thus help suppress or slow rates of invasion. Although overall native plant cover seems to

have the strongest influence on garlic mustard performance, managing for greater localized herb diversity may be important to achieve sufficiently high native plant cover levels.

The effects of light on garlic mustard invasion are somewhat complex, as they are mediated through native cover and garlic mustard seedling establishment. Plots with higher light levels had greater percent cover of native plants, which in turn exerted a negative influence on garlic mustard. Alone, this evidence would suggest that woodlands with high light availability would be more resistant to garlic mustard invasion, provided that native plants were present to respond to the available light. Light's strong negative effect on garlic mustard seedling establishment would seem to support this conclusion. However, due to apparent density dependence in garlic mustard, plots with higher light had fewer garlic mustard individuals, but greater total biomass and silique production per plot. In other words, higher light areas may tend to have higher native plant cover, and fewer but more productive garlic mustard individuals, while lower light areas may tend to have lower native plant cover, and more abundant but less productive garlic mustard. This may explain why garlic mustard populations are successful in both open woodlands and woodland edges (Meekins and McCarthy 2001), as well as in shadier areas and less-disturbed forest interiors (Nuzzo 1999).

What, then, can we infer about the combined effects of light, species richness and cover on long-term garlic mustard population dynamics? If fewer seeds establish in high light/high native cover areas, but they produce more siliques than the more abundant garlic mustard plants in low light/low cover areas, will there be any difference in the rates of garlic mustard population growth (i.e. rates of spread) with repeated iterations of this cycle over time? Although garlic mustard population modeling is beyond the scope of this article, we might speculate that two important factors in addressing this question are: 1) whether garlic mustard propagule pressure can overcome density-dependent effects on seedling establishment; and 2) the relative strength of the cover pathway and the light-cover-seedling pathway on total reproductive output.

Propagule pressure was not tested in this experiment, but others have found garlic mustard's self-pollination ability and high seed production to be important causal factors in its ability to establish new populations and spread rapidly (Anderson et al. 1996). In this experiment, I planted 50 garlic mustard seeds per plot, representing a seed rain of 200 seeds/m². But even after one generation, the seed rain increased substantially in most plots. Total silique production per plot ranged from 0 – 1512, with a mean of 389 (Table 3), or 0 – 6048 siliques/m² with an average of 1556 siliques/m². Average seed number per silique reported in the literature is approximately 13 (but can range from 1-30) (Trimbur 1973, Meekins and McCarthy 2002, Smith et al. 2003a, Evans and Landis 2007); so average estimated fecundity (seed/plant) in this study was greater than 5000 seeds/plant, and average estimated seed rain greater than 20,000 seeds/m², similar to reports of annual seed rain (15,000 seeds/m²) reported by Anderson (1996). Such considerable propagule pressure may overwhelm invasion resistance by native plant diversity and cover. Von Holle and Simberloff (2005) found that propagule pressure overwhelmed functional diversity-induced resistance to invasion in a riparian forest experiment. Clearly the role of propagule pressure in garlic mustard invasion and population dynamics warrants further attention, as it may influence which sites are most vulnerable to invasion.

However we might also consider the relative strength of the cover and the light-cover-seedling pathways on total silique numbers. In the SEM, the pathway with the strongest total effect on silique production per area was native plant cover (Figure 3; Table 4), indicating that native cover's suppression of garlic mustard reproduction may be stronger than the positive effects of light and density dependence (seedling number). This implies that, regardless of light levels, native plant cover is key to reducing rates of invasion. In fact, woodlands with high light levels and minimal native cover may experience the fastest rates of invasion, as high light may reduce garlic mustard seedling number, and the lack of both inter- and intra-specific competition for resources may greatly increase reproductive output. Thus when managing a woodland in a way that increases light levels, such burning (Bowles et al. 2007), canopy thinning and invasive

tree and shrub removal (Luken et al. 1997, Webb et al. 2001, Cipollini et al. 2009), it may be particularly important to include active native herb restoration to increase invasion resistance.

It is important to remember, though, that increasing invasion resistance does not mean preventing invasion. Woodlands with diverse and dense native groundcovers may be less hospitable for garlic mustard, but in this study even the highest levels of cover and diversity did not *prevent* establishment or maturity to reproduction. For example, plots with greater than 150% cover or greater than 6 native species per plot had fewer than five adult garlic mustard plants per plot in 2007. But garlic mustard is an obligate biennial (Byers and Quinn 1998) with the ability to self-pollinate (Trimbur 1973, Anderson et al. 1996, Cruden 1996), and as noted above even five garlic mustard plants can produce a substantial seed rain. A diverse native groundcover may slow the rate of garlic mustard spread, but is unlikely to prevent invasion.

Conclusion

Observed patterns of native decline and garlic mustard increase are often presented as evidence of garlic mustard's impacts on woodland plant communities. However, it is also possible that native plants affect garlic mustard invasion; that both garlic mustard and native plants influence each other; or that neither are directly interacting but instead responding in opposite directions to an external environmental change. This study does not support the latter hypothesis, as clearly native plants have a strong effect on garlic mustard in the early stages of invasion. It appears that garlic mustard may be responding to environmental change that causes native herb decline as opposed to, or in addition to, driving these changes in native woodland communities.

Native species richness and cover appear to have a strong influence on garlic mustard, therefore the loss of native plant richness and cover may contribute to invasion. As previously described, there are numerous reports of herb decline in North American woodlands (Brewer 1980, Robinson et al. 1994, Rooney et al. 2004, Rooney and Rogers

2011), and to some extent, garlic mustard's success in this region may be opportunistic, as it fills this vacated niche. Many of the same factors that cause herb decline may also be contributing to garlic mustard invasion. For example, deer may facilitate garlic mustard invasion by dispersing seeds (Anderson et al. 1996, Williams and Ward 2006), bringing buried seeds to the soil surface, and creating a disturbed microsite that is suitable for seedling establishment, in addition to preferential herbivory on garlic mustard's competitors (Nuzzo 1991, Eschtruth and Battles 2009a, Knight et al. 2009). Preferential herbivory on native herbs and avoidance of garlic mustard has also been reported in exotic slugs (Hahn et al. 2011). In heavily-used urban woodlands, humans likely also play a role in dispersing garlic mustard seeds and creating micro-disturbances that favor garlic mustard over native plants. The elimination of the duff layer by non-native earthworm invasion may also facilitate invasion of garlic mustard (Blossey et al. 2005, Nuzzo et al. 2009). Earthworms may also favor garlic mustard and other non-mycorrhizal plants by disrupting mycorrhizal mutualisms (Bohlen et al. 2004). In fact, Nuzzo et al. (2009) concluded that earthworms are the driving force of change in North American woodlands, with plant invasions and native herb decline being a response to this change. It seems likely that garlic mustard is benefitting doubly—both through direct facilitation by earthworms, and indirectly through the loss of native plants. All of these factors were abundant at the Warner Nature Center study site (pers. obs and Knight (2006)), however the study plots were fenced to prevent deer herbivory. Given the strong negative influence that over-abundant deer can have on native herbs, native plant communities may not achieve sufficient diversity and cover to suppress garlic mustard invasion without protection from deer herbivory.

Restoration of native woodland herbs may play an important role in resisting garlic mustard invasion (Bakker 2004, Corbin and D'Antonio 2004, Vidra et al. 2007), particularly in sites with high light availability. Light is clearly an important resource for the forest understory, although further research is needed to determine what additional factors (e.g. soil moisture, nutrients) may contribute to the influence of native plants on garlic mustard invasion. Other studies have found that communities with high or

fluctuating resource availability (Davis et al. 2000) and low native diversity or low capacity for natives to respond to increases in resource availability may be most susceptible to plant invasions (Byers and Noonburg 2003, Fridley et al. 2007), therefore in urban woodlands, where nutrient levels are often high and soil moisture generally is not limiting, restoring and maintaining a diverse native ground cover may be particularly important for limiting invasion. Restoration of native plants may slow garlic mustard invasion, but it will not prevent invasion. Even if only a few garlic mustard individuals establish, they are likely to survive and reproduce. However, if native plant cover slows the initial rate of invasion, as this study suggests, it may provide a longer window of time in which early detection and eradication measures are effective, when compared to sites that lack a native groundcover.

Table 4-1. Native species present in plots in 2007. Taxonomy follows USDA, NRCS 2011.

Planted in 2003 - 2004*	Colonized in 2006 - 2007
<i>Allium tricoccum</i> Aiton	<i>Amphicarpaea bracteata</i>
<i>Galium boreale</i> L.	<i>Laportea canadensis</i> (L.) Weddell
<i>Geranium maculatum</i>	<i>Parthenocissus</i> sp.
<i>Hydrophyllum virginianum</i> L.	<i>Pilea pumila</i> (L.) A. Gray
<i>Maianthemum canadense</i> Desf.	<i>Polygonum sagittatum</i> L.
<i>Maianthemum racemosum</i>	<i>Smilax ecirrhata</i> (Engelm. Ex Kunth) S. Watson.
<i>Phlox divaricata</i> L.**	<i>Stellaria media</i> ***
<i>Solidago flexicaulis</i> L.	Unidentified fern
<i>Symphotrichum cordifolium</i> (L.) G.L. Nesom**	
<i>Thalictrum dioicum</i> L.	
<i>Viola pubescens</i> Aiton	

* Species were planted as seedlings (plugs) purchased from Prairie Restorations, Inc., Princeton, MN.

** *S. cordifolium* experienced high mortality and was replaced with *P. divaricata* in some plots in 2004.

***Non-native species

Table 4-2. Average, standard error, range, and year(s) of measurement of native plant and environmental variables. Light and soil moisture data were obtained from Knight (2006); soil moisture was measured with a TDR (time domain reflectometry) device each summer from 2003-2005.

	Year	Mean	SE	Range
Native Plants				
Cover (%)	2005	55.1	6.4	0 - 187.1
Cover (%)	2007	79.2	9.4	0 - 250
Species Richness	2005	3.82	0.4	0 - 10
Species Richness	2007	2.92	0.27	0 - 8
Environmental Variables				
Light (DIFN)	2004-2005	0.13	0.004	0.09 - 0.21
Soil H ₂ O	2003-2005	14.54	0.51	5.7 - 23.3
pH (1:2)	2007	5.55	0.05	4.93 - 6.66
P (µg P/g resin/day)	2007	0.19	0.02	0.07 - 0.70
NO ₃ ⁻ (µg NO ₃ ⁻ /g resin/day)	2007	2.46	0.26	0.35 - 12.69
NH ₄ ⁺ (µg NH ₄ ⁺ /g resin/day)	2007	0.26	0.02	0.10 - 0.79

Table 4-3. Mean, standard error (SE) and range of garlic mustard response variables. Count data was used for the SEM. Percent data was calculated based on the number of seeds (out of 50) that produced seedlings, the number of rosettes that survived from the seedling stage, and the number of adults that survived from rosette, seedling, and seed stages.

Garlic Mustard	Year	Counts			Percent		
		Mean	SE	Range	Mean	Range	
GM Seedlings	2006	8.2	0.57	1 - 18	16.4	2 - 36	
Rosettes	2006	6.33	0.54	0 - 17	75.5	0 - 100	
Adults	2007	5.67	0.51	0 - 17	87.5	0 - 100	<i>from rosettes</i>
					69.2	0 - 100	<i>from seedlings</i>
					11.3	0 - 34	<i>from seeds</i>
Avg. Biomass/plant (g)	2007	10.96	1.84	0 - 77.15			
Total Biomass/plot (g)	2007	48.59	6.23	0 - 185.15			
Avg. Siliques/plant	2007	87.18	16.36	0 - 756			
Total Siliques/plot	2007	387.8	51.77	0 - 1512			

Table 4-4. SEM results summarizing the direct, indirect and total effects of light, native species richness and cover on garlic mustard invasion, including standardized estimates (Est.), standard error (S.E.) and statistical significance (p). The effects of garlic mustard seedling number on later life stages are also included. Standard errors are bootstrap estimates, and statistical significance is a bootstrap approximation from two-sided bias-corrected confidence intervals. Pathways indicated by estimates in bold font are statistically significant. Standardized effects can be interpreted as follows: when a factor increases by 1 standard deviation, the response variable changes by the number of standard deviations indicated by the estimate. For example, as light increases by 1 standard deviation, the total effect on garlic mustard seedling number is a decrease of 0.658 standard deviations. Average biomass per plant and average silique number per plant data were natural log-transformed. Total biomass and total silique number are per plot totals.

	Light			Richness			Cover			Seedling #		
	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect	Total
Seedling #												
Est.	-0.581	-0.077	-0.658	0.184	-0.183	0.001	-0.279		-0.279			
S.E.	0.07	0.032	0.063	0.134	0.09	0.113	0.115		0.115			
p	0.002	0.006	0.002	0.158	0.013	0.995	0.019		0.019			
Rosette #												
Est.	0.079	-0.639	-0.559	0.127	-0.264	-0.137	-0.403	-0.224	-0.627	0.802		0.802
S.E.	0.063	0.089	0.074	0.089	0.12	0.128	0.081	0.092	0.103	0.064		0.064
p	0.193	0.001	0.001	0.153	0.019	0.273	0.002	0.018	0.002	0.002		0.002
Adult #												
Est.	0.119	-0.539	-0.42	-0.139	-0.177	-0.316	-0.358		-0.358	0.734		0.734
S.E.	0.086	0.085	0.088	0.108	0.052	0.099	0.094		0.094	0.08		0.08
p	0.138	0.001	0.002	0.203	0.001	0.004	0.002		0.002	0.001		0.001
Avg. Biomass												
Est.	0.122	0.181	0.302	0.171	-0.36	-0.189	-0.697		-0.697	-0.405		-0.405
S.E.	0.159	0.115	0.159	0.133	0.083	0.135	0.14		0.14	0.116		0.116
p	0.469	0.167	0.069	0.195	<0.001	0.127	<0.001		<0.001	0.001		0.001
Total Biomass												
Est.	0.345	0.011	0.356	-0.156	-0.298	-0.453	-0.577		-0.577	-0.118		-0.118
S.E.	0.183	0.123	0.142	0.137	0.069	0.128	0.107		0.107	0.162		0.162
p	0.107	0.996	0.018	0.24	0.001	0.002	0.001		0.001	0.471		0.471
Avg. Silique #												
Est.	0.195	0.089	0.284	0.081	-0.347	-0.266	-0.701		-0.701	-0.308		-0.308
S.E.	0.146	0.129	0.158	0.141	0.086	0.135	0.155		0.155	0.128		0.128
p	0.187	0.53	0.063	0.595	<0.001	0.035	0.001		0.001	0.029		0.029
Total Silique #												
Est.	0.236	0.057	0.293	-0.115	-0.261	-0.375	-0.527		-0.527	-0.217		-0.217
S.E.	0.187	0.128	0.137	0.126	0.066	0.123	0.104		0.104	0.167		0.167
p	0.241	0.708	0.048	0.35	<0.001	0.002	0.001		0.001	0.227		0.227

Table 4-5. Chi-square, degrees of freedom, and p-values for each structural equation model. A non-significant p-value indicates that the data are consistent with the model.

Year	Garlic mustard responses	Chi-square	d.f.	p-value
First Year	Seedlings & Rosettes	0.537	1	0.464
Second Year	Adult numbers	3.515	4	0.476
Second Year	Average biomass per plant (ln)	3.297	4	0.509
Second Year	Total biomass per plot	3.297	4	0.509
Second Year	Average siliques per plant (ln)	3.515	4	0.476
Second Year	Total siliques per plot	3.515	4	0.476

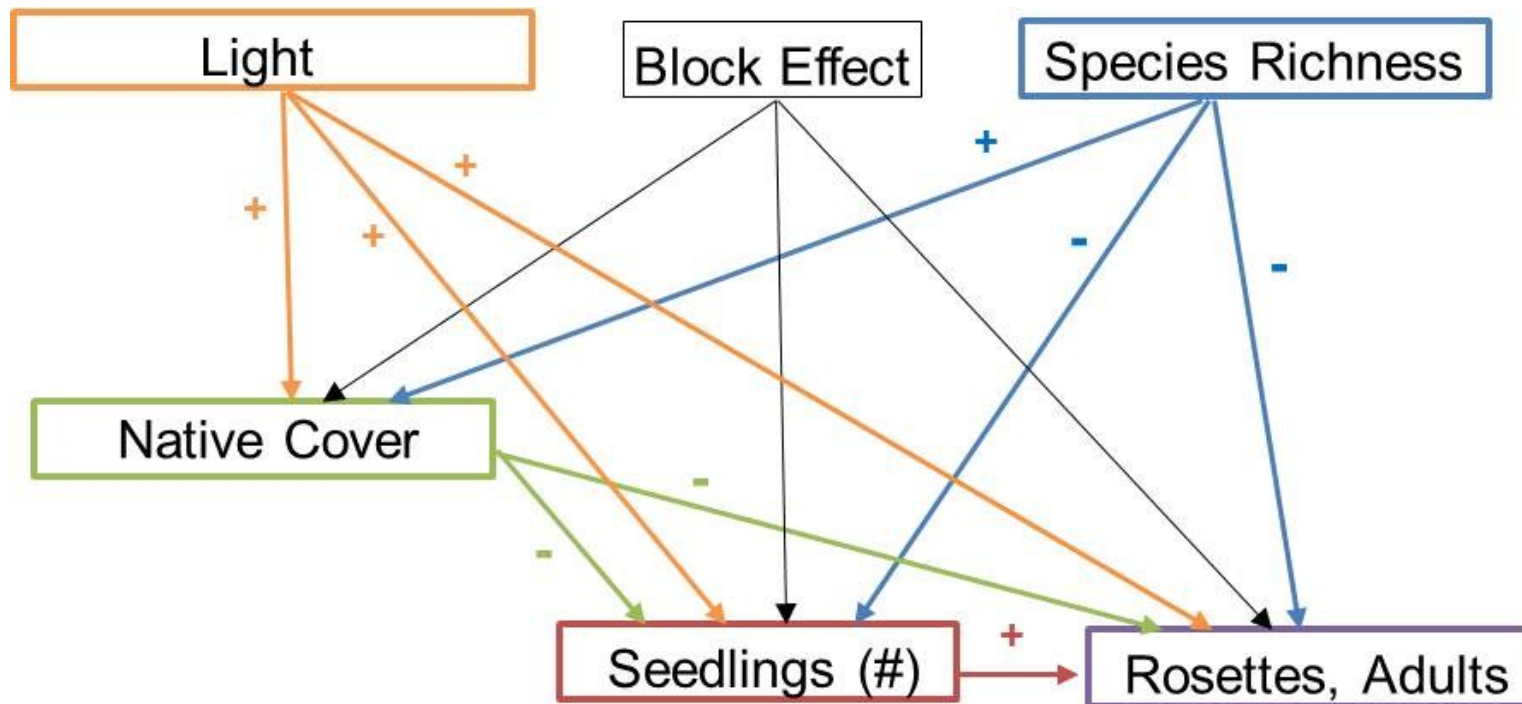


Figure 4-1. Hypothesized effects of light, native species richness and cover on garlic mustard responses. Light is expected to have a positive effect on native plant cover, as well as garlic mustard responses. Native cover and richness are expected to have a negative effect on garlic mustard responses, due to competitive effects. Seedling number is expected to have a positive effect on rosette and adult numbers, as well as total biomass and siliques per plot, but a negative effect on average biomass and siliques per plant (due to density-dependence). The block effect, which is defined primarily by NO_3^- and P, is included to statistically account for the grouping of subplots into “whole plots” (blocks). Both light and block effect are correlated with species richness.

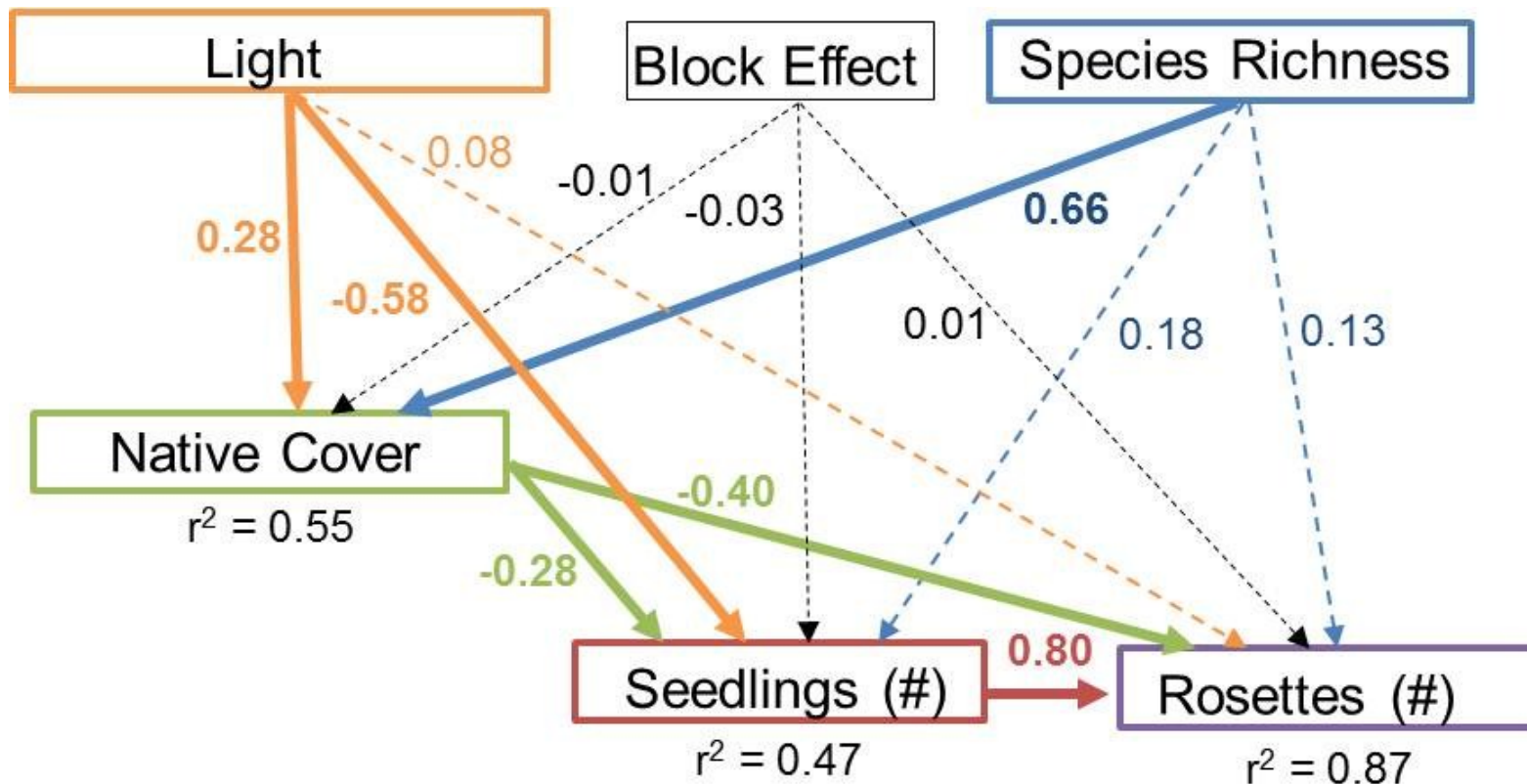


Figure 4-2. SEM depicting the relative effects of light, native plant cover and species richness on garlic mustard seedling establishment and rosettes (year 1). Solid arrows represent statistically significant pathways; dashed arrows represent non-significant pathways. Values adjacent to arrows indicate standardized estimates. Double-sided arrows are correlations. Light measurements were taken above the native vegetation.

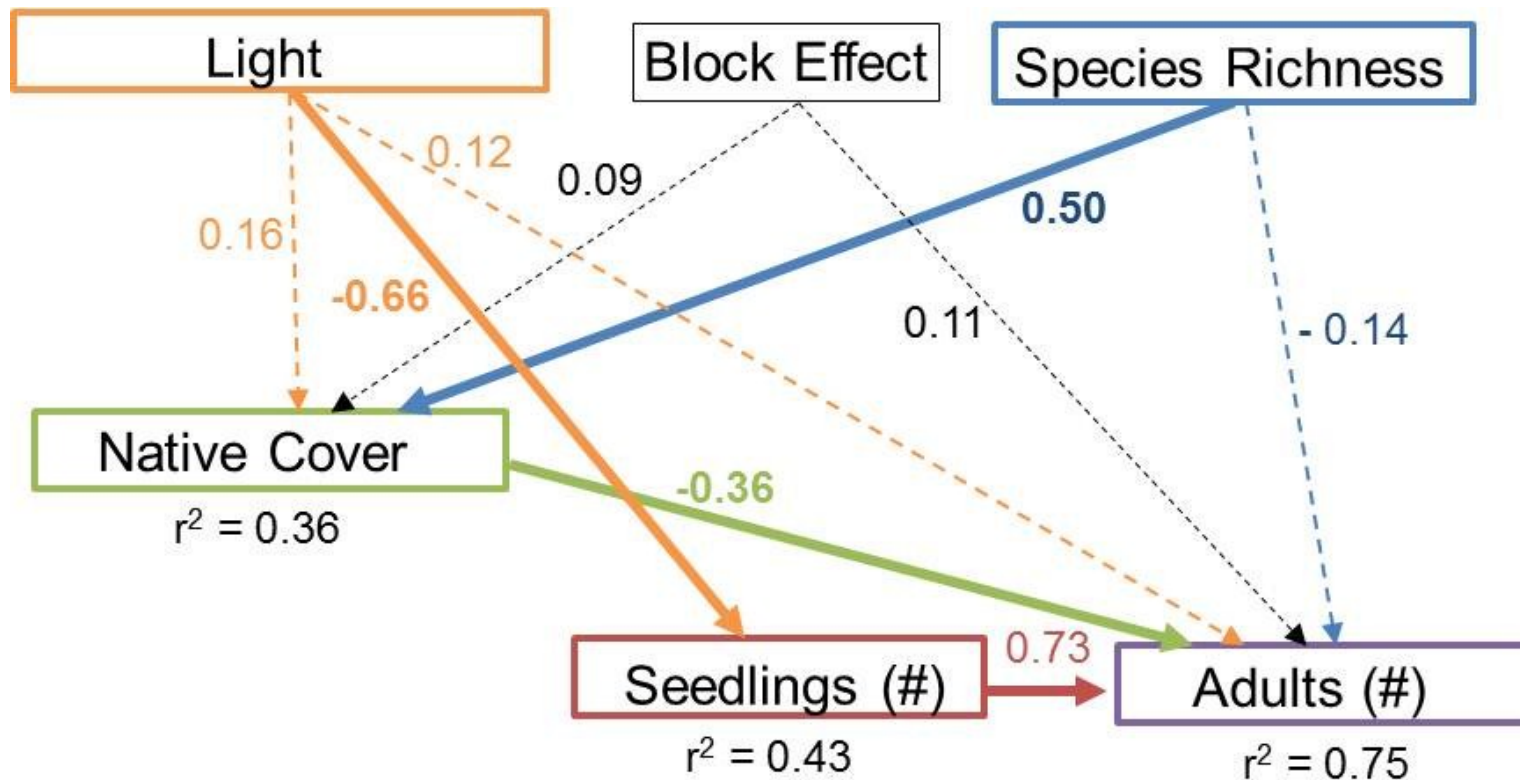


Figure 4-3. SEM depicting the relative effects of light, native plant cover and species richness, and number of garlic mustard seedlings (year 1) on the number of garlic mustard adults (year 2). Solid arrows represent statistically significant pathways; dashed arrows represent non-significant pathways. Values adjacent to arrows indicate standardized estimates. Double-sided arrows are correlations. Light measurements were taken above the native vegetation.

Chapter 5

Garlic Mustard Invasion, Impacts and Implications for Management and Restoration

Overview: Garlic Mustard Invasion and Impacts

The goal of this study was to evaluate the potential for restoring native herbs in a garlic mustard-invaded woodland. Specifically, I aimed to determine whether the presence or history of garlic mustard inhibits native herb restoration, and whether native herbs in turn affect the invasion success of garlic mustard. Woodland understory communities face numerous threats (Robinson et al. 1994, Rooney et al. 2004, Frelich et al. 2006, Hahn et al. 2011); garlic mustard, as a highly visible invader with prolific reproduction prolific seed producer (Trimbur 1973, Anderson et al. 1996), “novel weapons”(Callaway et al. 2008) and a dramatic rate of spread (Nuzzo 1999, Meekins and McCarthy 2002), is often implicated as a contributing factor in woodland decline, motivating extensive management and removal efforts (Nuzzo 1991) and a biocontrol research program (Blossey et al. 2001a, Van Riper et al. 2008). And yet, if garlic mustard is not a primary driver of change in North American woodlands, native communities may not recover despite garlic mustard control (Bauer 2012). Furthermore, if garlic mustard’s soil-mediated impacts persist after removal, even active attempts at restoration may be compromised.

Overall, my research results supported Bauer’s (2012) characterization of garlic mustard as a “back-seat driver” of change in woodland understory communities. Garlic mustard germination, survival, growth and reproduction were all influenced by native species richness and cover, suggesting that initial stages of invasion may be responding to—not causing—declines in native species. And although garlic mustard clearly exerts influence on soil chemistry and biota, the impacts on restored native herbs were not strongly or consistently negative, as would be expected if garlic mustard were driving

their decline. Restoration of native herbs may not be hindered by garlic mustard's presence or legacy effects, but management of other causes of woodland degradation will likely be necessary to ensure satisfactory restoration outcomes.

The greenhouse studies (Chapter 3) confirmed that garlic mustard can reduce AMF colonization rates in herb seedlings, as has been documented in tree seedlings (Stinson et al. 2006, Barto et al. 2011), but the reductions were minimal in the roots of herbs planted into woodlands, and not apparent in resident plants growing in invaded areas (Chapter 2). It may be that garlic mustard slows the initial rate of colonization in seedlings, but over multiple years in the field, root colonization in invaded areas eventually matches that of herbs in non-invaded areas, or the differences between the greenhouse and field studies may simply reflect different degrees of environmental variability in the controlled greenhouse experiments versus the field setting. Larger sample sizes for future field studies would be warranted, to more clearly determine the effects of invasion on herb colonization rates in a natural setting. Harvesting root samples at intervals across multiple herb life stages would also be informative in determining whether invasion effects on AMF colonization differs from seedling to mature plant.

Garlic mustard's impacts on native herbs were mixed. Most herb species had lower rates of germination in invaded soils, which raises concerns about long-term population trends. But both seedlings in the greenhouse and herbs planted as plugs or bare rootstock in the field generally had higher biomass in invaded soils compared to non-invaded soils. The net effect of invasion on herb population dynamics is unclear: if higher biomass correlates with greater reproductive output, this could potentially compensate for the reduced germination rates. To my knowledge, there are as of yet no published studies of garlic mustard's effects on native plant reproduction or on population dynamics. Such studies are needed to better predict the long-term effects of invasion on native plant communities.

The positive biomass response largely seemed to be explained by the higher nutrient availability in invaded soils, which persisted after multiple years of sustained garlic

mustard removal in the field. Nutrient leaching from the surrounding vegetation may have contributed to this apparent “positive legacy” and additional research on nutrient dynamics following broader-scale garlic mustard removal is needed to better understand the potential impacts for post-invasion restoration. Persistent facilitative effects on other species have been observed following removal of a nitrogen-fixing tree species in American Samoa (Hughes et al. 2012), while no lagged effect was observed following removal of the legume *Melilotus officinalis* in Great Plains grasslands (Van Riper 2004). The persistence of nutrient enhancement likely depends on the mechanism of enrichment (e.g. nitrogen fixation versus accelerated litter decomposition) and other ecosystem attributes. Rodgers et al. (2008b) first reported on garlic mustard’s ability to increase nutrient availability and suggested that it might create a positive feedback for garlic mustard invasion, yet the results of this research indicate that—when protected from herbivory—native plants may benefit from the fertilization effect as well. Under conditions of nutrient enrichment, AMF associations may either be irrelevant or even parasitic to host plants (Johnson et al. 1997, Rowe et al. 2007). The greenhouse study suggested that under ideal conditions, the AMF relationship might pose a net cost to the herbs, such that the combined effects of nutrient enrichment and reduced AMF might facilitate plant growth.

Native herbs did not always respond positively to garlic mustard-invaded soil, however. Contradictory responses to invasion in two replications of the greenhouse study suggest that the AMF-mediated effects of garlic mustard may become important in times of stress, possibly by increasing vulnerability of herbs to plant pathogens. The potential for pathogen accumulation in garlic mustard has not been reported aside from a symposium presentation that referenced unpublished data (Blossey et al. 2005), but additional investigation of the potential for complex interactions between garlic mustard, AMF and other organisms is clearly warranted. In a field study of the interactions between garlic mustard and exotic slugs, Hahn and Dornbush (2012) found that negative effects of garlic mustard were only apparent in plants that also experienced slug herbivory. Although my field studies did not find negative effects of garlic mustard

despite the presence of slugs, these experiments certainly raise interesting questions about how environmental conditions and additional stressors influence garlic mustard's impacts on native plants.

Several broad themes emerge from this research. First, the impacts of invasive species—and even the specific mechanisms of impact—are not uniform across native species, sites and environmental conditions. Just as previous research has shown native species to differ in their sensitivity to garlic mustard's competitive effects (Meekins and McCarthy 1999), this research suggests that species also differ in their sensitivity to garlic mustard's soil mediated effects, and that within-species, these impacts are contingent on environmental conditions and the presence of additional stressors. The findings that garlic mustard's AMF-mediated impacts may depend on environmental conditions highlights the need for a greater understanding of the plant-AMF relationship, particularly the conditions under which the relationship is beneficial or harmful to the host plant, as this adds complexity to our assumptions regarding the impact of invasive plants with anti-fungal properties. Additionally, the possibility that AMF impacts are counteracted, or even that the nature of the relationship is altered, via a separate nutrient enrichment pathway highlights the importance of examining the net effect of multiple simultaneous pathways of impact. Many invasive plants have been found to affect invaded communities via multiple mechanisms (Gordon 1998, Levine et al. 2003), and yet for experimental clarity, these mechanisms are typically tested individually. Doing so, however, may yield an incomplete picture of native plant responses to invasion.

It is also clear that invasion impacts need to be evaluated within a broader context of multiple ecosystem stressors. Rarely is a system affected by only a single invasive species or driver of environmental degradation; the potential for both counteracting effects and synergies between multiple stressors must be considered in order to predict community-wide impacts and develop comprehensive management plans. Individual native plants are likely to differ in their sensitivity to both the individual and combined effects of multiple stressors, creating the potential for “winners and losers” among native

plant species (McKinney and Lockwood 1999, Wiegmann and Waller 2006). In North American woodland communities, this likely means a shift in favor of species that are not obligately mycorrhizal, are tolerant of herbivory, and are adapted to both high nutrient availability and altered soil structure that results from earthworm invasion (Hale et al. 2005, Frelich et al. 2006). Reported increases in species like *Carex pennsylvanica* and *Arisaema triphyllum* suggest that this shift is already occurring (Bohlen et al. 2004, Holdsworth et al. 2007). Regional homogenization of plant communities is a current trend (Rooney et al. 2004) that will likely continue as globalization and climate change accelerate the introduction and spread of invasive species (Meyerson and Mooney 2007, Hellmann et al. 2008, Dukes et al. 2009).

Implications for Garlic Mustard Management and Woodland Restoration

Woodland herb restoration does not appear to be inhibited by garlic mustard's presence or its soil legacies. In fact, when protected from herbivory, herbs generally seemed to benefit from the nutrient enrichment effects of garlic mustard, particularly following garlic mustard removal. However, assessments of AMF colonization and garlic mustard removal studies suggest that native tree seedlings are more sensitive to garlic mustard's impacts than herbs (McCarthy 1997, Stinson et al. 2006, Stinson et al. 2007, Barto et al. 2011), therefore continued efforts to manage garlic mustard may be warranted. Because herbs are generally more tolerant of garlic mustard's impacts, they may play an important role in post-invasion woodland restoration.

Nutrient enrichment has been shown to favor exotic and ruderal species in many different ecosystems (Milchunas and Lauenroth 1995), thus garlic mustard removal without active restoration of native plants may open a window for invasion, creating a barrier to recovery of the native plant community. Native herbs are often dispersal- and recruitment-limited (Brudvig et al. 2011), and seeds may be particularly sensitive to garlic mustard's effects, so without planting, herbs may be unable to quickly respond to

the increased resource availability. Once restored, however, native herbs may limit the success of garlic mustard, potentially minimizing the opportunity for reinvasion. Although it seems likely that planting will be required to restore many invaded woodlands, field studies that evaluate the potential for natural recovery would be useful for assessing the trade-offs of cost, recovery time, and long-term outcomes associated with passive versus active restoration. Restoration by plugs or bare rootstock is recommended, as these plant forms appear to be less sensitive to garlic mustard's impacts than seeds, and the rapid establishment of native ground cover is important for utilizing available resources and preventing reinvasion. However, again, comparative analyses of the costs and effectiveness of woodland restoration strategies, particularly with regard to the effects of garlic mustard and other woodland stressors, would allow for more strategic restoration planning.

The restoration outlook for woodland herbs may not be entirely optimistic, however, for three main reasons. First, woodland herbs may have less reproductive success in garlic mustard-invaded areas as a result of lower germination rates and reduced seedling biomass in stressful climate conditions. Reductions in recruitment may have serious implications for long-term population viability, particularly in a changing climate. Drayton and Primack (2012) re-surveyed populations of restored native herbs 15 years after planting, and found that most populations had disappeared—even those that had appeared to be viable a few years after planting. They caution against claiming restoration “success” until multiple viable generations are established. Thus the long-term viability of restored herbs, including those in my field study, is not guaranteed by initial survival. Long-term population monitoring of woodland herb communities are necessary to understand the trajectories of plant community composition in response to invasion and its control.

A second reason for caution is that the apparent positive response of herbs to garlic mustard-affected soils may only be experienced in the absence of herbivory and other environmental stressors. While the loss of AMF may not “matter” to native herbs in a

climate-controlled greenhouse or when protected from herbivory in the field, this pattern may not hold in less-ideal conditions. Under stress, herbs may in fact be compromised by persistent effects of garlic mustard. Given the documented impacts of deer, slugs and earthworms on native plants, this certainly raises concerns about the restoration potential in many North American woodlands. Climate change may further exacerbate negative impacts via the loss of AMF. In Minnesota, woodlands are expected to experience hotter and drier summer conditions (Galatowitsch et al. 2009); under these conditions, AMF may be more important to native herbs, and the effects of garlic mustard may shift from positive to negative. Coupled with garlic mustard's negative effects on germination, this may bode ill for long-term population dynamics of native herbs.

A final serious concern regarding post-invasion restoration of woodlands is that controlling garlic mustard alone is not likely reverse woodland decline. As a “back-seat driver” of change, garlic mustard's impacts appear to merely contribute to declines that are already occurring. Unless primary drivers of change are identified and mitigated, even active restoration may fail to result in successful or sustainable outcomes. In North American prairies, fire suppression is a recognized driver of change, shifting plant communities toward dominance by exotic cool-season grasses and invading shrubs (Briggs et al. 2005). Restoration of prairies thus generally involves a comprehensive approach of removing the undesirable vegetation, planting native species and addressing the driving force of change by reintroducing fires when possible (Rowe 2010). A similar comprehensive approach to woodland restoration is needed. Currently, woodland understory management is often limited to controlling invasive plants, with minimal replanting of herbs and no real strategy for mitigating the driving forces of change. Increasingly, invasive earthworms are being implicated as driver of change in North American woodlands (Bohlen et al. 2004, Frelich et al. 2006, Nuzzo et al. 2009), but as of yet, there is no method for controlling earthworms or managing their impacts, other than attempting to prevent their spread. White-tailed deer (*Odocoileus virginianus* Zimmerman) herbivory is also known to have considerable impacts on forest understory plants (Rooney and Waller 2003, Côté et al. 2004, Webster et al. 2005, Wiegmann and

Waller 2006) yet social and political barriers exist to more intensive deer management. Invasive plant species, such as garlic mustard, buckthorn (*Rhamnus cathartica*) and honeysuckle (*Lonicera* spp.), are—in contrast—very visible, their control is relatively uncontroversial (particularly garlic mustard, which has little horticultural value), and management options do exist, labor-intensive though they may be. Continued garlic mustard control may be a component of a comprehensive approach to woodland management and restoration, but will not likely be sustainable or effective in the long run without creative plans for mitigating the effects of earthworms, deer, and future climate change. However, if forthcoming biocontrol is successful, this may free up resources that could then be allocated toward native plant restoration and continued research and management of the primary drivers of woodland change.

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Appendix A: Chapter 2 Anova Tables

Environmental Variables

Light (above-plot)

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	41.4650	41.4650	60.71	<.0001
Invs	1	1.2392	1.2392	1.81	0.18
Site*Invs	1	1.1973	1.1973	1.75	0.19

Light (forest floor) at CG

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Light_above	1	1.5774	1.5774	5.84	0.02
Invs	1	0.0023	0.0023	0.01	0.93
Removal	1	0.7479	0.7479	2.77	0.10
Invs*Removal	1	0.1829	0.1829	0.68	0.42
Light_above*Invs	1	0.1113	0.1113	0.41	0.52
Light_above*Removal	1	5.1601	5.1601	19.1	<.0001
Light_above*Invs*Removal	1	0.0105	0.0105	0.04	0.84

Light (forest floor) at WNC

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Light_above	1	0.0688	0.0688	0.27	0.61
Invs	1	0.2833	0.2833	1.1	0.31
Light_above*Invs	1	0.2107	0.2107	0.82	0.38
Removal	1	0.9632	0.9632	3.73	0.07
Light_above*Removal	1	0.0026	0.0026	0.01	0.92
Invs*Removal	1	0.5131	0.5131	1.99	0.18
Light_above*Invs*Removal	1	0.3820	0.3820	1.48	0.24

Soil Texture - % Sand

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	3089.0250	3089.0250	120.47	< 0.0001
Invs	1	18.7911	18.7911	0.73	0.40
Site*Invs	1	36.4495	36.4495	1.42	0.24

Soil Texture - % Clay

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	59.3476	59.3476	24.55	< 0.0001
Invs	1	2.7192	2.7192	1.12	0.30
Site*Invs	1	7.5581	7.5581	3.13	0.08

Soil Texture - % Silt

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	2292.0406	2292.0406	132.41	< 0.0001
Invs	1	7.2140	7.2140	0.42	0.52
Site*Invs	1	10.8119	10.8119	0.62	0.43

Soil Moisture

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	30.9191	30.9191	308.52	< 0.0001
Invs	1	0.5441	0.5441	5.43	0.02
Site*Invs	1	0.0530	0.0530	0.53	0.47
Removal	1	0.0316	0.0316	0.32	0.58
Site*Removal	1	0.2334	0.2334	2.33	0.13
Invs*Removal	1	0.0101	0.0101	0.1	0.75
Site*Invs*Removal	1	0.0001	0.0001	0	0.98
PlantYr	1	0.1602	0.1602	1.6	0.21
Site*PlantYr	1	0.0122	0.0122	0.12	0.73
Invs*PlantYr	1	0.0203	0.0203	0.2	0.65
Site*Invs*PlantYr	1	0.0242	0.0242	0.24	0.62
Removal*PlantYr	1	0.2372	0.2372	2.37	0.13
Site*Removal*PlantYr	1	0.1264	0.1264	1.26	0.26

Soil pH

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	0.1801	0.1801	84.26	<.0001
Invs	1	0.0024	0.0024	1.13	0.29
Removal	1	0.0008	0.0008	0.39	0.54
Site*Invs	1	0.0006	0.0006	0.28	0.60
Site*Removal	1	0.0001	0.0001	0.06	0.81
Invs*Removal	1	0.0029	0.0029	1.38	0.25
Site*Invs*Removal	1	0.0081	0.0081	3.79	0.06

Phosphorus

(P) May – July 2007

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	0.0874	0.0874	0.36	0.55
Invs	1	6.4545	6.4545	26.38	<.0001
Site*Invs	1	2.0022	2.0022	8.18	0.01
Removal	1	0.7216	0.7216	2.95	0.09
Site*Removal	1	0.0278	0.0278	0.11	0.74
Invs*Removal	1	0.0957	0.0957	0.39	0.54
Site*Invs*Removal	1	0.2066	0.2066	0.84	0.36

(P) August – September 2007

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	0.2476	0.2476	0.72	0.40
Invs	1	2.8015	2.8015	8.12	0.01
Site*Invs	1	0.0407	0.0407	0.12	0.73
Removal	1	0.6114	0.6114	1.77	0.19
Site*Removal	1	0.0438	0.0438	0.13	0.72
Invs*Removal	1	0.0917	0.0917	0.27	0.61
Site*Invs*Removal	1	0.0539	0.0539	0.16	0.69

(P) May – August 2008

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	1.9703	1.9703	5.47	0.02
Invs	1	8.3011	8.3011	23.06	<.0001
Site*Invs	1	1.1246	1.1246	3.12	0.08
Removal	1	0.0061	0.0061	0.02	0.90
Site*Removal	1	1.3785	1.3785	3.83	0.05
Invs*Removal	1	0.0193	0.0193	0.05	0.82
Site*Invs*Removal	1	1.3652	1.3652	3.79	0.05

Nitrate

(NO₃⁻) May – June 2007

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	4.7742	4.7742	9.35	0.004
Invs	1	0.7621	0.7621	1.49	0.23
Site*Invs	1	5.4173	5.4173	10.61	0.002
Removal	1	0.8021	0.8021	1.57	0.22
Site*Removal	1	1.4025	1.4025	2.75	0.11
Invs*Removal	1	0.5473	0.5473	1.07	0.31
Site*Invs*Removal	1	2.5078	2.5078	4.91	0.03

(NO₃⁻) August – September 2007

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	8.6715	8.6715	14.72	0.00
Invs	1	14.3049	14.3049	24.28	<.0001
Site*Invs	1	3.0805	3.0805	5.23	0.03
Removal	1	0.5912	0.5912	1	0.32
Site*Removal	1	0.4270	0.4270	0.72	0.40
Invs*Removal	1	0.0659	0.0659	0.11	0.74
Site*Invs*Removal	1	0.1884	0.1884	0.32	0.58

(NO₃⁻) May – August 2008

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	1.6656	1.6656	2.97	0.09
Invs	1	9.2764	9.2764	16.52	0.0002
Site*Invs	1	1.2936	1.2936	2.3	0.14
Removal	1	2.7948	2.7948	4.98	0.03
Site*Removal	1	0.2966	0.2966	0.53	0.47
Invs*Removal	1	1.7115	1.7115	3.05	0.09
Site*Invs*Removal	1	0.1564	0.1564	0.28	0.60

Ammonia

(NH₃⁺) May – July 2007

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	0.3533	0.3533	0.48	0.49
Invs	1	3.5853	3.5853	4.83	0.03
Site*Invs	1	6.7337	6.7337	9.07	0.005
Removal	1	0.3801	0.3801	0.51	0.48
Site*Removal	1	1.3004	1.3004	1.75	0.19
Invs*Removal	1	1.5339	1.5339	2.07	0.16
Site*Invs*Removal	1	0.1645	0.1645	0.22	0.64

(NH₃⁺) August – September 2007

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	5.5025	5.5025	16.84	0.0002
Invs	1	0.0406	0.0406	0.12	0.73
Site*Invs	1	0.2300	0.2300	0.7	0.41
Removal	1	0.9699	0.9699	2.97	0.09
Site*Removal	1	4.1982	4.1982	12.85	0.0009
Invs*Removal	1	0.2092	0.2092	0.64	0.43
Site*Invs*Removal	1	0.1621	0.1621	0.5	0.49

(NH₃⁺) May – August 2008

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	0.0000	0.0000	0	0.99
Invs	1	4.9560	4.9560	6.29	0.02
Site*Invs	1	2.3301	2.3301	2.96	0.09
Removal	1	0.8879	0.8879	1.13	0.29
Site*Removal	1	0.3064	0.3064	0.39	0.54
Invs*Removal	1	2.4780	2.4780	3.14	0.08
Site*Invs*Removal	1	0.0631	0.0631	0.08	0.78

Biomass Measurements

Three-year-old plants

WNC/CG Species at CG

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	18	1.34	0.26
Removal	1	18	2.39	0.14
Invs*Removal	1	18	0.63	0.44
Species	5	275	36.31	<.0001
Invs*Species	5	275	2.64	0.02
Removal*Species	5	275	1.27	0.28
Invs*Removal*Species	5	275	2.26	0.05
Light_above*Removal	2	275	1.73	0.18

WNC/CG Species at WNC

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	18	1.68	0.21
Removal	1	18	1.12	0.30
Invs*Removal	1	18	0.66	0.43
Species	5	246	3.49	0.005
Invs*Species	5	246	2.76	0.02
Removal*Species	5	246	2.82	0.02
Invs*Removal*Species	5	246	1.3	0.26
Light_above	1	246	6.25	0.01
Light_above*Removal*Species	11	246	2.2	0.01

(Three-year old) CG-only Species

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	17	0.67	0.42
Removal	1	17	0.17	0.68
Invs*Removal	1	17	0.12	0.74
Species	2	158	6.93	0.001
Invs*Species	2	158	7.26	0.001
Removal*Species	2	158	4.91	0.01
Invs*Removal*Species	2	158	0.27	0.76
Light_above	1	158	0.05	0.83
Light_above*Invs	1	158	0.92	0.34
Light_above*Removal	1	158	0.3	0.58
Light_above*Species	2	158	0.13	0.88
Light_above*Invs*Species	2	158	7.48	0.0008
Light_above*Removal*Species	2	158	3.42	0.04

Two-year-old plants; analysis with light covariate

WNC/CG Species at CG

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	16	13.43	0.002
Removal	1	16	2.67	0.12
Invs*Removal	1	16	3.14	0.10
Species	7	335	3.94	0.00
Invs*Species	7	335	4.89	<.0001
Removal*Species	7	335	2.49	0.02
Invs*Removal*Species	7	335	1.98	0.06
Light_above	1	335	3.2	0.07
Light_above*Species	7	335	2.3	0.03

(Two-year-old) WNC/CG Species at WNC

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	17	1.15	0.30
Removal	1	17	1.63	0.22
Invs*Removal	1	17	0.31	0.59
Species	7	232	4.17	0.0002
Invs*Species	7	232	0.67	0.69
Removal*Species	7	232	2.53	0.02
Invs*Removal*Species	7	232	0.95	0.47
Light_above	1	232	2.19	0.14
Light_above*Removal	1	232	0.16	0.69
Light_above*Species	7	232	1.14	0.34
Light_*Removal*Species	7	232	2.67	0.01

CG-only Species

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	16	12.56	0.003
Removal	1	16	0.05	0.83
Invs*Removal	1	16	0.71	0.41
Species	2	113	83.36	<.0001
Invs*Species	2	113	4.98	0.01
Removal*Species	2	113	1.6	0.21
Invs*Removal*Species	2	113	6.63	0.002
Light_above	1	113	0.45	0.50

Two-year-old plants; analysis with light & nutrient covariates

WNC/CG Species at CG

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	14	0.91	0.36
Removal	1	14	7.94	0.01
Invs*Removal	1	14	0.1	0.76
Species	7	328	4.93	<.0001
Invs*Species	7	328	0.62	0.74
Removal*Species	7	328	2.78	0.01
Invs*Removal*Species	7	328	2.15	0.04
Light_above	1	328	4.59	0.03
Light_above*Species	7	328	2.43	0.02
N_axis	1	328	5.04	0.03
N_axis*Removal	1	328	4.84	0.03
N_axis*Species	7	328	3.04	0.004

WNC/CG Species at WNC

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	14	1.61	0.23
Removal	1	14	2.81	0.12
Invs*Removal	1	14	0	0.98
Species	7	233	4.18	0.0002
Invs*Species	7	233	0.67	0.70
Removal*Species	7	233	2.58	0.01
Invs*Removal*Species	7	233	0.97	0.46
Light_above	1	233	2.96	0.09
Light_above*Removal	1	233	0.23	0.63
Light_above*Species	7	233	1.15	0.33
Light_*Removal*Species	7	233	2.75	0.01
N_axis	1	233	4.16	0.04
N_axis*Invs	1	233	3.88	0.05

(Two-year-old) CG-only Species

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	15	3.58	0.08
Removal	1	15	0.09	0.77
Invs*Removal	1	15	0.97	0.34
Species	2	113	83.32	<.0001
Invs*Species	2	113	4.89	0.01
Removal*Species	2	113	1.58	0.21
Invs*Removal*Species	2	113	6.54	0.002
Light_above	1	113	0.47	0.49
N_axis	1	113	1.72	0.19

Degree of Removal Analysis

Three-year-old plants at CG

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Removal	2	14	1.28	0.31
Species	5	209	38.42	<.0001
Removal*Species	10	209	1.54	0.13
Light_above	1	209	4.85	0.03

Three-year-old plants at WNC

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Removal	2	14	1.29	0.31
Species	5	189	58.2	<.0001
Removal*Species	10	189	0.79	0.63
Light_above	1	189	15.2	0.0001

Two-year-old plants at CG; plots with three years of removal

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Removal	2	13	0.75	0.49
Species	7	283	36.79	<.0001
Removal*Species	14	283	1.05	0.40
Light_above	1	283	0.52	0.47

Two-year-old plants at WNC; plots with three years of removal

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Removal	2	13	2.61	0.11
Species	7	155	2.57	0.02
Removal*Species	14	155	0.95	0.50
Light_above	1	155	14.78	0.0002
Light_above*Removal	2	155	1.57	0.21
Light_above*Species	7	155	5.35	<.0001
Light_above*Removal*Species	14	155	0.75	0.72

Two-year-old plants at CG; plots with two years removal

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Removal	2	14	1.77	0.21
Species	7	297	34.07	<.0001
Removal*Species	14	297	1	0.45
Light_above	1	297	5.19	0.02

Two-year-old plants at WNC; plots with two years removal

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Removal	2	14	4.27	0.04
Species	7	176	24.92	<.0001
Removal*Species	14	176	1.95	0.02
Light_above	1	176	11.54	0.0008

Duration of Removal Analyses

Site: CG – with light covariate

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
YrsRem	2	8	2.45	0.15
Species	7	195	27.24	<.0001
YrsRem*Species	14	195	1.62	0.08
Light_above	1	195	2.72	0.10

(Duration) Site: WNC – with light covariate

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
YrsRem	2	13	7.67	0.01
Species	7	172	35.45	<.0001
YrsRem*Species	14	172	1.32	0.20
Light_above	1	172	9	0.003

Site: CG – analysis with light & nutrient covariates

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
YrsRem	2	6	5.78	0.04
Species	7	187	29.9	<.0001
YrsRem*Species	14	187	1.73	0.05
Light_above	1	187	5.92	0.02
N_axis	1	187	7.83	0.01
N_axis*YrsRem	2	187	3.35	0.04
N_axis*Species	7	187	2.93	0.01

Site: WNC– analysis with light & nutrient covariates

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
YrsRem	2	13	6.21	0.01
Species	7	171	35.65	<.0001
YrsRem*Species	14	171	1.27	0.23
Light_above	1	171	7.72	0.01
N_axis	1	171	0.33	0.57

AMF Analysis – Restoration Study

WNC/CG Species

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Site	1	21	1.27	0.27
Invs	1	21	0.05	0.82
Site*Invs	1	21	3.70	0.07
Species	3	51	31.98	<.0001
Site*Species	3	51	2.68	0.06
Invs*Species	3	51	2.38	0.08
Site*Invs*Species	3	51	0.15	0.93

CG-only Species

<u>Effect</u>	<u>Num DF</u>	<u>Den DF</u>	<u>F Value</u>	<u>Pr > F</u>
Year	1	35	0.76	0.39
Invs	1	20	1.88	0.19
Year*Invs	1	35	0.12	0.73
Species	2	35	15.50	<.0001
Year*Species	2	35	0.71	0.5
Invs*Species	2	35	0.86	0.43
Year*Invs*Species	2	35	0.41	0.67

AMF Analysis – Resident Plants

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.0004	0.0004	0.01	0.90
Species	2	0.3697	0.1848	6.72	0.002
Species*Invs	2	0.0398	0.0199	0.72	0.49

Appendix B: Chapter 3 Anova Tables

Germination Study

Germination percent

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Species	7	4.7250	0.6750	97.58	<.0001
Invs	1	0.0348	0.0348	5.04	0.03
Species*Invs	7	0.0675	0.0096	1.39	0.22

Germination timing

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Species	7	10.5710	1.5101	25.57	<.0001
Invs	1	0.1709	0.1709	2.89	0.09
Species*Invs	7	0.6978	0.0997	1.69	0.13

Seedling Establishment

Biomass – Rep. 1

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.0209	0.0209	5.01	0.03
Fung	1	0.1550	0.1550	37.22	<.0001
Species	6	4.2489	0.7082	170.08	<.0001
Invs*Fung	1	0.0099	0.0099	2.37	0.12
Invs*Species	6	0.0107	0.0018	0.43	0.86
Fung*Species	6	0.0759	0.0126	3.04	0.01
Invs*Fung*Species	6	0.0486	0.0081	1.95	0.07

Biomass – Rep. 2

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.1015	0.1015	8.10	0.005
Fung	1	0.1108	0.1108	8.84	0.003
Species	6	6.9823	1.1637	92.87	<.0001
Invs*Fung	1	0.0338	0.0338	2.70	0.10
Invs*Species	6	0.1828	0.0305	2.43	0.02
Fung*Species	6	0.1685	0.0281	2.24	0.04
Invs*Fung*Species	6	0.0230	0.0038	0.31	0.93

Root Fraction – Rep. 1

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.1547	0.1547	16.24	<.0001
Fung	1	0.0939	0.0939	9.86	0.002
Species	6	4.6198	0.7700	80.81	<.0001
Invs*Fung	1	0.0122	0.0122	1.28	0.26
Invs*Species	6	0.6065	0.1011	10.61	<.0001
Fung*Species	6	0.2712	0.0452	4.74	0.0001
Invs*Fung*Species	6	0.0885	0.0148	1.55	0.16

Root Fraction – Rep. 2

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.0886	0.0886	6.08	0.01
Fung	1	0.0227	0.0227	1.56	0.21
Species	6	10.3225	1.7204	118.00	<.0001
Invs*Fung	1	0.0017	0.0017	0.11	0.74
Invs*Species	6	0.0470	0.0078	0.54	0.78
Fung*Species	6	0.0678	0.0113	0.77	0.59
Invs*Fung*Species	6	0.0250	0.0042	0.29	0.94

AMF – Rep. 1

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.4708	0.4708	16.28	0.0001
Fung	1	0.1572	0.1572	5.43	0.02
Species	1	0.0015	0.0015	0.05	0.82
Invs*Fung	1	0.0768	0.0768	2.66	0.11
Invs*Species	1	0.0417	0.0417	1.44	0.23
Fung*Species	1	0.0004	0.0004	0.01	0.91
Invs*Fung*Species	1	0.0020	0.0020	0.07	0.79

AMF – Rep. 2

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.0740	0.0740	2.71	0.10
Fung	1	0.0364	0.0364	1.33	0.25
Species	1	0.2343	0.2343	8.57	0.005
Invs*Fung	1	0.0764	0.0764	2.79	0.10
Invs*Species	1	0.2163	0.2163	7.91	0.01
Fung*Species	1	0.0008	0.0008	0.03	0.86
Invs*Fung*Species	1	0.0822	0.0822	3.01	0.09

Non-AM fungi – Rep. 1

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	2.8193	2.8193	58.65	<.0001
Fung	1	0.0106	0.0106	0.22	0.64
Species	2	0.6758	0.3379	7.03	0.002
Invs*Fung	1	0.1027	0.1027	2.14	0.15
Invs*Species	2	1.0619	0.5309	11.04	<.0001
Fung*Species	2	0.2082	0.1041	2.17	0.12
Invs*Fung*Species	2	0.0620	0.0310	0.64	0.53

Non-AM fungi – Rep. 2

<u>Effect</u>	<u>DF</u>	<u>Type III SS</u>	<u>Mean Square</u>	<u>F Value</u>	<u>Pr > F</u>
Invs	1	0.0001	0.0001	0.02	0.89
Fung	1	0.0571	0.0571	8.37	0.005
Species	2	0.0387	0.0193	2.83	0.06
Invs*Fung	1	0.0131	0.0131	1.91	0.17
Invs*Species	2	0.0298	0.0149	2.18	0.12
Fung*Species	2	0.0309	0.0155	2.27	0.11
Invs*Fung*Species	2	0.0098	0.0049	0.72	0.49